MicroRNA-148a facilitates inflammatory dendritic cell differentiation and autoimmunity by targeting MAFB

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Abstract
Monocyte-derived dendritic cells (moDCs) have been implicated in the pathogenesis of autoimmunity, but the molecular pathways determining the differentiation potential of these cells remain unclear. In this paper, we report that microRNA (miR)-148a serves as a critical regulator for moDC differentiation. Firstly, miR-148a deficiency impaired the moDC development in vitro and in vivo. Following mechanism study manifested that MAFB, a transcription factor that hampers moDC differentiation, was a direct target of miR-148a. In addition, promoter study further identified that miR-148a could be transcriptionally induced by PU.1, which is crucial for moDC generation. MiR-148a ablation eliminated the inhibition of PU.1 on MAFB. Furthermore, we found that miR-148a increased in monocytes from psoriasis patients, and miR-148a deficiency or intradermal injection of antagomir-148a immensely alleviated the development of psoriasis-like symptoms in a psoriasis-like mouse model. Therefore, these results identify a pivotal role for PU.1-miR-148a-MAFB circuit in moDC differentiation and suggest a potential therapeutic avenue for autoimmunity.
Introduction

Dendritic cells (DCs) comprise a heterogeneous family of professional antigen presenting cells (APCs) that maintain the homeostasis of immune system (1-4). DC populations in the skin have been well investigated in mice (5-7). In steady state, two major subsets of conventional DCs (cDCs) have been characterized in the mouse non-lymphoid tissues, CD103⁺CD11b⁻ cDCs and CD103⁻CD11b⁻ cDCs, whereas the epidermis contains Langerhans cells (LCs). In inflammatory state, peripheral monocytes are rapidly recruited to inflammatory sites and give rise to monocyte-derived DCs (moDCs) (5-7). Moreover, plasmacytoid DCs (pDCs), a potent type I interferon producing DC subset, are also documented increased frequency and activation status in early psoriatic lesions (8, 9). Both cDCs and pDCs are originated from DC precursors in a Fms-related tyrosine kinase 3 ligand (Flt3L) dependent manner, while the generation of moDCs is granulocyte-macrophage colony stimulating factor (GM-CSF) dependent (1, 2).

Up to now, the regulation of moDC activation in autoimmune diseases has been extensively studied (10-13). Transcriptional factors, such as STAT5, PU.1 and MAFB, have already been demonstrated to affect moDC generation at different stages of hematopoietic differentiation (14-20). Both PU.1 and MAFB involve in the myeloid commitment of hematopoietic stem cells, and the balance between them specifies alternative DC or macrophage fate (18, 19). Despite intensive study, the molecular mechanism underlying regulatory network for moDC generation is far from clarified.

Psoriasis is a common, chronic auto-inflammatory skin disease characterized by demarcated, red and scaly plaques. Environmental and genetic factors trigger the hyperproliferation and disturbed differentiation of keratinocytes, thickening of the epidermis, formation of new blood vessels and accumulation of leukocytes in epidermis and dermis, of which T cells and DCs are the most critical (21, 22). Psoriatic skin contains large numbers of activated DCs, which produce IL23, IL12, IL6, and TNFα. They activate and polarize auto-aggressive helper T (Th) cells toward Th17, Th1 and Th22 cell subsets defined by the production of IL17A, IL17F, IFNγ and IL22,
respectively (9, 23, 24). Recently, the involvement of different skin DC subsets in regulating psoriatic plaque formation has been extensively studied. PDCs and cDCs are dispensable for the local skin inflammation in psoriasis, since \( E2-2^{+/} \) mice and \( Flt3L^{-/-} \) mice presented no significant difference in ear swelling and epidermal thickening when compared with the wild type (WT) mice (25, 26). The \( Ccr2^{-/-} \) mice, which have profound monocytopenia due to a defect in monocyte egress from bone marrow (BM) showed significantly reduced skin pathological phenotype (26). Depletion of monocytes in vivo by using antibody also greatly relieved the symptoms (26). The function of LCs is controversial since Lan-DTR mice developed a similar degree and course of psoriasiform skin disease as those of WT mice in an imiquimod (IMQ) induced model (25), while it showed a certain degree of improvement in IL23 induced model (26). The finding that locally increased expression of GM-CSF and IL23 was exclusively produced by Langerin⁺ DCs in vivo, which further supports moDCs as the most important DC subset in psoriasis (25).

MiRNAs are short (~22 nt), evolutionarily conserved, single-stranded RNAs that control the expression of complementary target mRNAs, leading to their transcript destabilization, translational inhibition, or both (27). MiRNAs are critical regulators of immune cell development and function (28). In this study, we identified \( miR-148a \) as a paramount regulator for autoimmune related moDC differentiation by using the psoriasis disease model. \( MiR-148a \) deficiency led to decreased pathogenic moDCs. Consequently, either \( miR-148a \) knockout or \( miR-148a \) inhibition by intradermal administration with antagonimr-148a, prevented the development of moDCs and psoriasis-like inflammation in the IMQ-induced psoriasis-like mouse model. The mechanism research revealed that \( MAFB \) was a bona fide direct target of \( miR-148a \), which was transcriptionally regulated by PU.1. Therefore, these results identify a pivotal role for PU.1-miR-148a-MAFB pathway in the differentiation of moDCs and suggest a potential therapeutic avenue for inflammatory moDCs mediated autoimmune diseases.
Results

MiR-148a is indispensable for moDC differentiation.

Pro-inflammatory moDC infiltration is critical for the progression of several autoimmune diseases, including systemic lupus erythematosus (SLE), psoriasis, inflammatory bowel disease (IBD) and so on (25, 29, 30). A comprehensive analysis of our previous data in patients with SLE (31) or psoriasis revealed an increase in the expression of miR-148a-3p, (named ‘miR-148a’ here) (Figure S1). Other groups also reported similar elevation in autoimmune diseases (32). Therefore, we hypothesized that miR-148a might affect the development or function of pathogenic moDCs in autoimmune diseases.

Before evaluating the function of miR-148a in psoriatic inflammation, we firstly checked the effect on hematopoietic differentiation in steady state (Figure S2A-D). Accordingly, different cell subsets in the spleen and skin from miR-148a−/− and WT littermate mice were detected, respectively (Figure S2A and S2C). Consequently, there was no difference in the number of lymphoid or myeloid cells (Figure S2B and S2D), indicating that miR-148a was dispensable for hematopoietic differentiation in steady state.

In inflammatory state, Ly6C hi monocytes migrate to the lesion sites and then differentiate into moDCs. To validate whether the DC differentiation from monocyte was affected by miR-148a, Ly6C hi monocytes were isolated from BM of miR-148a−/− and WT mice, and then cultured with GM-CSF and IL4. It has been demonstrated that Ly6C hi monocytes can give rise to both CD11c+MHCII hiCD11b int DC and CD11c+MHCII intCD11b hi macrophages (10). The conclusion was confirmed in the culture system that sorted CD11c+MHCII hiCD11b int cells exhibited DC morphology and CD11c+MHCII intCD11b hi cells had a typical macrophage morphology (Figure 1A and 1B), allowing us to evaluate the differentiation potential of monocytes towards macrophages and DCs in the same culture system.

There was no difference of the expanding potential of monocytes between control and miR-148a deficiency, since the number of live cells was similar (Figure 1C). MiR-148a−/− monocytes gave rise to considerably less moDCs (about 70% reduction) than those of controls (Figure 1A and 1C). In contrast, both the percentage and number of macrophages increased in miR-148a−/− group (Figure 1A and 1C), indicating a critical
role for miR-148a in promoting the differentiation of monocytes toward inflammatory DCs at the expense of macrophage.

Previous studies reported that miR-148a was involved in anti-apoptosis, and frequently upregulated in primary and tumor cells (33, 34). To confirm whether the decrease in the proportion and number of miR-148a−/− moDCs was resulted from the increased apoptosis or not, the entire cells were stained with annexin V and propidium iodide (PI). Consequently, the examination of cell apoptosis showed no difference between miR-148a−/− and WT throughout the inducing process, suggesting that miR-148a didn’t affect the moDC survival (Figure 1D).

MAFB is a direct target of miR-148a.

To gain insight into the molecular mechanism and thus facilitate the differentiation of moDCs for miR-148a, we performed RNA-sequencing analysis of monocytes isolated from WT mice or miR-148a−/− mice in the presence of GM-CSF and IL4, or not. Gene ontology analysis showed that obviously changed genes enriched in autoimmune disease related pathways (SLE, RA, et al.), suggesting miR-148a was important in autoimmune response (Figure 2A). Since miRNA promotes the degradation of its target gene mRNAs, the mRNA abundance of miR-148a target gene should be higher in miR-148a−/− monocytes than that in WT monocytes. The threshold was set at ±0.2 CT, since the degrading potential of target mRNA by miRNA is much weaker than siRNA. Then we identified 990 protein-coding genes upregulated in their mRNA abundance in miR-148a−/− monocytes on day 0, and 1036 genes in miR-148a−/− monocytes on day 3 (Figure 2B). Among them, we found that 136 genes were upregulated on both day 0 and 3 in miR-148a−/− monocytes. Bioinformatic analysis finally helped confirm that 7 out of 136 genes contained predicted conserved miR-148a binding sites (Figure 2B). Targets previously implicated in hematopoietic differentiation were of special interest because the results suggested a role for miR-148a in regulating moDC generation. Of these 7 genes, MAFB has been reported to increase from monocyte to macrophage otherwise decrease from monocyte to moDC (18, 20). Overexpression of MAFB in myeloid progenitor cells is proposed to induce macrophage differentiation at the expense of
These resulted in the selection of MAFB for further study (Figure 2C).

The RNA-sequencing results were validated by qRT-PCR (Figure S3A), and the protein expression encoded by MAFB was examined by immunoblot (Figure 2D). Both the mRNA and protein levels of MAFB increased in miR-148a–/– monocytes. We transfected human primary monocytes with antagonir-148a as well. The results showed that knockdown of miR-148a led to the increase of MAFB expression (Figure S3D).

To test the direct binding of miR-148a with MAFB mRNA, we performed the RIP assay through using an antibody against Argonaute-2 (Ago2), a component of the RNA-induced silencing complex (RISC) that mediates miRNA-directed gene silencing. The results showed that overexpression of miR-148a significantly enriched the association of MAFB mRNA with the Ago2-containing complex, suggesting that the MAFB mRNA was directly bound by miR-148a (Figure 2E). Furthermore, to confirm the effect of miR-148a on the MAFB 3′UTR, dual-luciferase reporter assays were conducted. Gene fragments encoding 3′UTR region of MAFB were cloned into the expression vector psiCHECK2, which encodes the renilla luciferase controlled by the cloned 3′UTR. These constructs were co-transfected in HEK 293T cells with miR-148a mimic. Data showed that miR-148a overexpression significantly reduced reporter protein expression through the 3′UTR region of MAFB (Figure 2F). When the predicted binding site for miR-148a was mutated, the reduction was abolished (Figure 2F). Therefore, miR-148a can suppress the expression of MAFB through its cognate binding site in 3′UTR.

Since miR-148a directly regulates MAFB expression, it is crucial to confirm that the higher protein level of MAFB mediates the miR-148a–/– phenotype. To this end, miR-148a–/– and WT monocytes were transfected with specific siRNA for MAFB, and then cultured with GM-CSF and IL4. Knockdown of MAFB promoted the moDC differentiation potential of miR-148a–/– monocytes comparable with that of the WT monocytes (Figure 2G, S3B and S3C). These data therefore demonstrated that MAFB mediated the phenotype of miR-148a deficiency and confirmed that MAFB was a bona fide direct target of miR-148a.
MiR-148a is involved in the inhibition of MAFB expression by PU.1.

Considering that miR-148a can regulate moDC differentiation, we then explored to determine how this miRNA was transcriptionally regulated. Previous studies have identified the transcriptional start sites (TSSs) of miR-148a (35). Intriguingly, a conserved PU.1 binding site was identified in the promoter (Figure 3A). To validate whether PU.1 can modulate miR-148a expression, we transfected primary monocytes with PU.1 siRNA. Knockdown of PU.1 led to the reduction of miR-148a expression (Figure 3B, S4A and S4B). To further explore the physiological relevance of PU.1-dependent regulation of miR-148a and subsequent MAFB expression, we measured the dynamic changes of PU.1, miR-148a and MAFB during moDC differentiation. As expected, miR-148a level paralleled with the increase in PU.1 mRNA and protein levels, otherwise for the MAFB mRNA and protein levels (Figure 3C, 3D and S4C).

To further determine whether miR-148a participated in the inhibition of MAFB expression by PU.1, both miR-148a−/− and WT monocytes were transfected with PU.1 siRNA. The results manifested that the inhibitory effect of PU.1 on MAFB expression disappeared in the miR-148a−/− monocytes (Figure 3E, S4D and S4E). Therefore, miR-148a was indispensable for the inhibition of MAFB expression by PU.1.

PU.1 directly activates miR-148a promoter.

To investigate the role of PU.1 in directly regulating miR-148a expression, we generated luciferase reporter constructs containing a 200bp region of the human miR-148a promoter. As shown in Figure 3F, overexpression of PU.1 enhanced miR-148a promoter activity compared with that for cells transfected with an empty vector. Besides, deletion of the PU.1 binding site significantly attenuated the promoter activity.

To determine whether PU.1 binds to this site in monocytes, we performed the ChIP assays. PU.1-chromatin complexes were immunoprecipitated from monocytes with a PU.1 monoclonal antibody, and quantitative PCR with site-specific primers was designed to analyze for enrichment of the identified site. Clear PU.1 occupancy was observed for the putative binding site within the promoter region of miR-148a (Figure 3G).
In summary, these researches demonstrated that PU.1 inhibited MAFB expression through transcriptional activation of *miR-148a* directly.

**MiR-148a deficiency alleviates the development of psoriasis.**

To investigate the functional relevance of *miR-148a* in the development of moDCs, we subjected *miR-148a*−/− mice to the IMQ-induced psoriasis-like mouse model. Repetitive application of IMQ onto WT mouse skin led to psoriasiform inflammation with significant thickening, redness, and scaling caused by keratinocyte hyperproliferation and leukocyte infiltration into the skin (Figure 4A-4F). In contrast, *miR-148a*−/− mice developed much less skin inflammation, exhibiting less thickening, redness, scaling (Figure 4A), ear thickness (Figure 4B) and psoriasis area and severity index (PASI) score (Figure 4C). H&E-stained back skin sections of *miR-148a*−/− mice displayed a reduction in dermal thickness and acanthosis (Figure 4D-F) during the course of disease. On day 6, the peak of psoriasis-like changes, we observed that the splenomegaly of mice in *miR-148a*−/− group was much less obvious than that in control group (Figure S5A and S5B). In accordance with this, the reduced mRNA of *IL23, TNFα, IL6, IL1β*, and *IL17a* was observed in the skin lesions of the *miR-148a*−/− group (Figure 4G). Meanwhile, the protein levels of IL23, IL1β, and IL17a were also found to be remarkably decreased in the serum of *miR-148a*−/− mice (Figure 4G).

To further determine the inflammatory cell infiltration in dermis from the *miR-148a*−/− group, we obtained dermal single-cell suspensions and analyzed the percentage of different immune cell subsets. The percentage of CD45+ immune cells, including moDCs (CD45+MHCIImiLy6C−CD64−CD11c+CD11b+) and IL17+ γδT cells (CD45+CD3+ γδTCR+IL17A+), reduced in dermis greatly (Figure S5C, 4H and 4I). In contrast, macrophages (CD45+MHCIIMiLy6C+CD64+) increased (Figure 4H and 4I). Similarly, the number of splenocytes, B cells, Ly6Chi monocytes, moDCs, CD4+ T cells, CD8+ T cells, pDCs and CD8α- cDCs in spleen decreased in *miR-148a*−/− group with IMQ application (Figure S5B and S5D). Meanwhile, both CD8α+ cDCs and neutrophils in spleen were not affected (Figure S5D). To determine whether moDCs reduced in the skin lesions of *miR-148a*−/− mice, we also performed the immunochemistry (IHC) assay.
on skin sections through using an antibody specific for mouse CD11c. The results showed that moDCs decreased in the dermis of miR-148a<sup>−/−</sup> mice with respect to those of WT mice (Figure S5E). Furthermore, we also detected the expression of MAFB and found that MAFB decreased in this model (Figure S5F).

In conclusion, these results suggested a reduction of systemic inflammation in miR-148a<sup>−/−</sup> group during the psoriasis.

**MiR-148a in moDC contributes to inflammation.**

To confirm whether miR-148a exerted effect on psoriasis through depending on immune cells rather than other skin tissue cells, WT or miR-148a<sup>−/−</sup> BM cells were transplanted into sub-lethally irradiated CD45.1<sup>+</sup> recipients, respectively, to generate BM chimeric mice. Six weeks after transplantation, these mice were subjected to the IMQ-induced psoriasis-like mouse model (Figure S6A). MiR-148a<sup>−/−</sup> chimeric mice developed much less skin inflammation, exhibiting less thickening, redness, scaling (Figure S6B), PASI score and ear thickness (Figure S6C). Hematoxylin and eosin (H&E) stained back skin sections of miR-148a<sup>−/−</sup> chimeric mice displayed a reduction in acanthosis and dermal thickness (Figure S6D and S6E) compared with those of control. Meanwhile, we observed that the splenomegaly of mice in miR-148a<sup>−/−</sup> chimeric group was much less obvious than that in control group (Figure S6F-S6H). FACs analysis of dermis showed that miR-148a<sup>−/−</sup> chimeric mice had much less infiltration of CD45<sup>+</sup> immune cells, including moDCs and IL17<sup>+</sup> γδT cells (Figure S6I). Similarly, the reduced mRNA levels of IL23a, TNFα, and IL17a were also observed in the skin lesions of the miR-148a<sup>−/−</sup> chimeric group (Figure S6J).

To further address that the role of miR-148a was moDC intrinsic, we adopted antibody mediated depletion of monocytes in both miR-148a<sup>−/−</sup> and WT mice. The available antibodies allowed us to deplete Ly6C<sup>hi</sup> monocytes plus neutrophils or neutrophils alone (Figure 5A and S7A). Depleting neutrophils alone had little effect on the inflammatory response and psoriasis progression (Figure 5B-5G), which was consistent with previous report (26). The additional depletion of Ly6C<sup>hi</sup> monocytes led to a partial loss of infiltrating monocytes and moDCs (Figure 5G and S7A),...
accompanied with significant decrease in ear swelling, PASI score (Figure 5C), dermal thickness, acanthosis (Figure 5D and 5E), and mRNA levels of IL23a, TNFα, IL6, and IL17a (Figure 5F). More crucial, there was no difference in the severity of psoriasis, like skin inflammation between IMQ painted miR-148a−/− and WT mice after Ly6Chi monocyte depletion, since they showed similar reduction in ear and epidermal thickness (Figure 5B-5E), and in moDC and IL17+ γδT cell infiltration (Figure 5G). The difference in mRNA level of the inflammatory cytokines also greatly diminished (Figure 5F). In accordance with these, the difference in splenomegaly and moDC number between WT and miR-148a−/− mice disappeared in monocyte depletion group (Figure S7B and S7C). Consequently, the fact that depletion of monocytes eliminated the acceleratory effects of miR-148a on the development of psoriasis, suggested that the role of miR-148a was more likely moDC intrinsic.

**Intradermal administration of antagonim-148a ameliorates the pathological phenotype of IMQ-induced psoriasis-like mice.**

The results above indicated that the elevated miR-148a expression could promote psoriasis pathogenesis. Therefore, we next sought to evaluate the efficacy and potential application of a miR-148a-based therapy for psoriasis. Antagomir-148a or antagomir-NC were intradermally injected 4 times from the beginning to the third day in order to test the inhibitory effect on disease development in this model (Figure 6A). MiR-148a expression in skin lesions dramatically reduced (Figure S8A). The results displayed a significant improvement in both clinical and pathological characteristics on day 5 following antagomir-148a treatment (Figure 6B-6E), along with alleviated disease severity (Figure 6C) and splenomegaly (Figure S8B and S8C). In addition, IMQ-treated mice injected with antagonim-148a manifested lower level of IL23a, TNFa, IL6, IL1β and IL17a mRNA in skin lesions (Figure 6F). We also assessed the effect of antagonim-148a in vivo on moDCs and IL17+ γδT cells in psoriasis-like mouse models. Silencing of miR-148a significantly reduced the percentage of moDCs and IL17+ γδT cells in skin lesions (Figure 6G and 6H). In accordance with the decrease of MAFB in IMQ-induced
model (Figure S5F), mice with antagomir-148a administration showed higher MAFB level than those with antagomir-NC (Figure S8D).

Since it has been demonstrated that miR-148a could regulate moDC differentiation by directly targeting MAFB in vitro, it is crucial to confirm the mechanism in vivo. Accordingly, miR-148a/− and WT mice were administrated with specific siRNA for MAFB or NC, and painted with IMQ for 6 days. Then the knockdown efficiency was verified (Figure S9A). Compared with the mice with NC siRNA, those with MAFB siRNA showed a significant aggravation in both clinical and pathological characteristics (Figure S9B-E), along with exacerbated disease severity (Figure S9C) and splenomegaly (Figure S9F). Furthermore, MAFB knockdown weakened the effect of miR-148a deficiency, as the difference between WT and miR-148a/− mice narrowed in MAFB siRNA treated group, compared with NC treated groups (Figure S9B-F). Thus, MAFB partially mediated the function of miR-148a in vivo.

Finally, in order to examine the relevance between miR-148a level and psoriasis disease, we detected the expression of miR-148a in peripheral monocytes from psoriasis patients. Compared with those from healthy controls, the mRNA levels of IL23a, IL1β and IL17a increased in psoriatic PBMCs (Figure 7A). Monocytes from psoriasis patients showed the increased miR-148a level (Figure 7B). The expression of miR-148a positively correlated with the mRNA levels of IL23a and IL17a (Figure 7C), supporting that miR-148a was crucial in psoriasis. To confirm the PU.1-miR-148a-MAFB circuit in psoriasis in vivo, we detected the expression of PU.1 and MAFB in peripheral monocytes from psoriasis patients. The results demonstrated that monocytes from psoriasis patients showed decreased MAFB expression but the increased PU.1 level (Figure 7D and 7E). Moreover, the expression of miR-148a positively correlated with the mRNA level of PU.1 (Figure 7E), while negatively correlated with MAFB level (Figure 7D).

Consequently, these results indicated that the inhibition of miR-148a expression in vivo could prevent the immunopathological changes in psoriasis and effectively ameliorate disease severity, suggesting a novel treatment strategy for psoriasis and other autoimmune diseases.
Discussion

The transcriptional regulation of moDC differentiation has been extensively studied (2, 13). The balance of MAFB and PU.1 was considered to specify alternative macrophage or DC fate (18). The differentiation of monocytes to DCs results in a rapid increase of PU.1 achieving high levels that precedes phenotypic changes, otherwise decrease of MAFB expression. Inhibited MAFB expression in monocytes appears to be required for DC specification, since constitutive MAFB expression inhibits DC differentiation (18). In addition, MAFB deficiency is found specifically to enhance sensitivity to M-CSF and cause activation of the myeloid master-regulator PU.1 in HSCs in vivo (19).

One possible mechanism for PU.1 regulation of MAFB function is that PU.1 can directly bind to MAFB and inhibit MAFB transactivation capacity. In this research, we found that miR-148a mediated the inhibition of MAFB expression through being regulated by PU.1, suggesting a new mechanism for regulating the balance of MAFB and PU.1, and thereby the differentiation of moDCs. Interestingly, a recent study reported that PU.1 could directly bind to the super-enhancer of miR-148a and facilitate its maturation in mouse primary B cells (36). This remains to be elucidated whether this regulation exists in primary monocytes and moDCs.

MiRNA is generally regarded as a multifunctional molecule and can exert diverse roles in different immune cells by targeting different molecules intracellularly (28). Increased expression of miR-148a, which occurs frequently in lupus patients and lupus-prone mice, impaired B cell tolerance through promoting the survival of immature B cells upon B cell receptor engagement via suppressing the expression of GADD45a, PTEN and BCL2l11, and facilitated the development of lethal autoimmune disease (33). MiR-148a could also considerably reduce the DNMT1 protein level, which was one of the major epigenetic components that linked to DNA hypomethylation in T cells in SLE (31). There was no significant difference in the number of B cell and T cell subsets between miR-148a-/- mice and WT mice in the steady state. Hence, miR-148a might affect the survival, function of both autoreactive B and T cells in inflammatory state, respectively. In order to confirm the cell intrinsic role of miR-148a in moDCs, we
designed two experiments. Firstly, we constructed BM chimeric mice and found that miR-148a<sup>−/−</sup> chimeric mice demonstrated less psoriatic skin inflammation, indicating that miR-148a only takes effect on immune cells rather than other skin tissue cells during the process. Secondly, monocytes were depleted in vivo through using antibodies before and during IMQ painting. Monocytes depletion greatly alleviated the severity of the psoriasiform skin inflammation. In addition, there was no significant difference in the symptoms between miR-148a<sup>−/−</sup> mice and WT mice, indicating that miR-148a in moDCs is more likely critical for the IMQ induced psoriasis-like inflammation.

Besides, miR-148a has also been reported to regulate the innate immune response. It was demonstrated to negatively regulate TLR4-triggered functional maturation of moDCs by targeting CaMKIIα (34), and inhibit macrophage function by directly targeting several upstream regulators of NF-κB and STAT3 signaling, including GP130, IKKα, IKKβ, IL1R1 and TNFR2 (37). We also found that miR-148a<sup>−/−</sup> moDCs could produce increased levels of TNFα and IL6 in response to LPS stimulation (data not shown). However, less than 10% increase was rather limited when compared with the decreased potential of moDC differentiation both in vitro (about 70% reduction) and in vivo (more than 1-fold reduction) as shown in Figure 1C, 4I and 6H. Therefore, miR-148a in monocytes exerted deleterious effects during psoriasis.

In addition to the miR-148a, several other miRNAs have been reported to regulate the differentiation and function of moDCs, such as miR-21, miR-34a and miR-155 etc (38-41). Besides miR-148a, both miR-21 and miR-34a also affect moDC generation, and miR-155 promotes the apoptosis and activation of moDCs. As molecule fine-tuning gene expression, these miRNA-targets generate a complex regulatory network comprised of different levels, including miR-21/WNT1 and miR-34a/JAG1 at the ligand/receptor level, miR-155/SOCS1 at the signal transduction level and miR-148a/MAFB at the transcriptional factor level. Interestingly, miR-21 was found to be abnormally elevated in psoriasis, and the inhibition has been shown to have a beneficial effect on the treatment of psoriasis (42). In addition, miR-155 is also upregulated in
psoriatic biopsy samples and responsible for maintaining the inflammation in psoriasis (43).

In conclusion, the present study provides evidence that miR-148a, which could be transcriptionally activated by PU.1, promotes moDC differentiation through directly targeting MAFB, thereby causing the immune imbalance and inflammatory response in skin lesions of psoriasis (Figure 8). Since inflammatory DCs are pathogenic in various autoimmune diseases (SLE, RA, etc.), this research suggests that miR-148a could be served as a potential target for the treatment of not only psoriasis, but also other autoimmune diseases.
Methods

Human Subjects. A total of 34 psoriatic patients who were diagnosed with psoriasis vulgaris were recruited from outpatient clinics in Huashan Hospital of Fudan University. Psoriasis disease activity was assessed through using PASI score. The patient information is shown in Supplemental Table 1. In addition, a total of 51 sex- and age-matched healthy controls were recruited from Renji Hospital of Shanghai Jiao Tong University School of Medicine.

Mice. All animal experiments were authorized by the Animal Care Committee of Renji Hospital. The C57BL/6J mice were acquired from Slack Company. MiR-148a KO mice B6/JNju-Mir148aem2Cd262in1/Nju with the C57BL/6 background were provided by Model Animal Research Center, Nanjing University. In addition, male mice at 6 to 8 weeks of age were employed to carry out all the following experiments. All mice were bred and housed under the conditions of specific pathogen-free (SPF) circumstance.

IMQ-induced psoriasis-like mouse model and antagonim-148a and MAFB siRNA treatment. Male miR-148a−/− mice with C57BL/6J background (6-8 weeks of age) were daily painted with IMQ cream (5%) (Aldara; 3M Pharmaceuticals) on the shaved back for the indicated days, and then sacrificed for the subsequent experiments. Besides, the control mice were applied with the same dose of vehicle cream. Male miR-148a−/− mice and their littermates (6-8 weeks of age) were injected intradermally with antagonim-148a (10nM in 150 µL PBS) or antagonim-NC (10nM in 150 µL PBS) on day 0, 1, 2 and 3 during the application of IMQ (44). Antagonim-148a (GenePharma, B05001) and the corresponding negative control antagonim-NC (GenePharma, B04007) were purchased from Shanghai GenePharma Co., Ltd. As for MAFB siRNA treatment, male miR-148a−/− mice and their littermates (6-8 weeks of age) were administrated intradermally with siRNA specific for MAFB (10nmol in 100 µL PBS ) or NC (10nmol in 100 µL PBS) on day 0, 1, 2, and 3 during the application of IMQ. In addition, MAFB
siRNA (Ambion, 4457308) and NC siRNA (Ambion, 4457289) were purchased from Applied Biosystems Inc.

**Scoring severity of skin inflammation in psoriasis-like mouse model.** An objective scoring system was developed based on the clinical PASI for assessing the psoriasis patients. Accordingly, erythema, thickening, and scaling were scored according to the scope, respectively, from 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked. The overall score was obtained by calculating a sum of the 3 index scores (score 0-12).

**Cell isolation, flow cytometry analysis, and cell culture.** Skin cells were prepared on the basis of the previous researches with minor modifications (45). In brief, the epidermis and dermis were separated by adopting the dispase (25U/ml in DPBS), and then the dermal cell suspension were acquired through using the collagenase and hyaluronidase. Splenocytes were prepared through digesting the tissue fragments with collagenase IV and DNase I. Antibodies employed for cell staining were presented in Supplemental Table 2. Cells were analyzed through using Fortessa (BD) and FlowJo software.

PBMCs were separated from the peripheral blood of healthy controls and psoriasis patients by density gradient centrifugation (GE Healthcare). Human and mouse monocytes were isolated with the Human Monocyte Isolation Kit II and Mouse Monocyte Isolation Kit (BM) (Miltenyi, Germany), respectively. Then the cells were cultured in RPMI 1640 medium (Gibco) with 10% fetal bovine serum (FBS, Gibco) at 37°C and 5% CO₂, or collected for subsequent experiments. HEK 293T cells and HeLa cells were maintained in DMEM (Gibco) supplemented with 10% FBS (Gibco). To obtain moDCs, BM cells or Ly6C^hi^ monocytes separated from BM were cultured with 100ng/mL GM-CSF and 100ng/mL IL4 (Peprotech) for indicated days. The Annexin V Apoptosis Detection Kit (BioLegend) were used for apoptosis assays according to the manufacturer’s protocol.
**SiRNAs and agomir transfection.** BM cells, mouse monocytes isolated from BM or human monocytes isolated from peripheral blood were transfected with Lipofectamine RNAiMAX (Invitrogen) according to the protocol. Hela cells and HEK 239T cells were transfected with Lipofectamine 3000 (Invitrogen). Briefly, small interfering RNA (siRNA; 200nM), agomir (200nM), plasmids and transfection reagents were diluted with Opti-MEM (Gibco) and then mixed gently. After incubation for 15 minutes at room temperature, the transfection mixture was added to the cell culture. Cells were harvested for subsequent experiments. The agomir-148a (GenePharma, B06002) and the corresponding negative control (GenePharma, B04008) were purchased from Shanghai GenePharma Co., Ltd. Human *PU.1* siRNA (Santa Cruz, sc-36330), mouse *PU.1* siRNA (Santa Cruz, sc-36331) and *MAFB* siRNA (Santa Cruz, sc-35840) were purchased from Santa Cruz Biotechnology Inc.

**Histology.** As for hematoxylin and eosin staining, the histological analysis was conducted as Lai Y, *et al.* reported (46). Firstly, mouse skin tissues were fixed in formalin and embedded in paraffin, and then stained with hematoxylin and eosin (H&E). Finally, the dermal thickness was assessed by measuring the thickness from the stratum papillare to the stratum reticulare and epidermal hyperplasia from the basal layer to the stratum corneum by using Case Viewer software (3DHISTECH Ltd). The measurement of skin thickness was conducted on 6 randomly selected areas per x10 field from 3 to 5 fields per section. The obtained skin thickness was firstly averaged per mouse and then employed to calculate the average per treatment group for the following statistical analysis. Scale bar indicates 100µm. As for immunochemistry, the mouse skin paraffin sections were stained with hematoxylin and mouse monoclonal anti-CD11c antibody (GB11059, Servicebio). In addition, the scale bar indicates 50µm.

**Construction of BM chimeras.** B6.CD45.1 recipients were sub-lethally irradiated by X-ray (8Gy), and then intravenously transferred with 5 x 10^6 BM cells derived from *miR-148a^-/-* mice or C57BL/6J mice. Chimeras were adopted for experiments 6 weeks after the initial reconstitution.
Depletion of neutrophils and monocytes. The depletion in vivo was conducted based on the report by Singh et al. (26). In brief, as for the depletion of neutrophils alone, the mice were administrated intraperitoneally with 500 mg antibody anti-Ly6G (clone 1A8, BioXCell, West Lebanon, NH, USA) on day 0, 1, 2, 3 and 4, respectively. Meanwhile, in order to deplete monocytes and neutrophils, the mice were injected intraperitoneally with 500 mg of antibody anti-Gr1 (clone RB6-8C5, BioXCell, West Lebanon, NH, USA) at the same timepoint as above.

mRNA-Seq and miRNA-Seq. All the RNA was firstly extracted by employing the miRNeasy Micro Kit (Qiagen, Hilden, Germany) and determined with Bioanalyzer 4200 (Agilent, Santa Clara, CA, USA). Then the next-generation mRNA libraries were prepared with VAHTS mRNA-seq v2 Library Prep Kit for Illumina® (Vazyme, Nanjing, China), while miRNA libraries with NEBNext® Multiplex Small RNA Library Prep Set for Illumina® (NEB, USA) and then purified by QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany). Afterwards, the acquisitions from above were subsequently put for size selection through employing the gel Prep. Besides, the Library quality was determined by Bioanalyzer 4200 (Agilent, Santa Clara, CA, USA). Then the mRNA-seq libraries were run in HiSeq X10 system (Illumina, San Diego, CA, USA) on a 150-bp paired-end run, otherwise the miRNA-seq libraries were sequenced on an Illumina HiSeq 2500 next generation sequencer using Single-read 50-bp sequencing. Among the compared groups, those with more than 1.2-fold difference of the geometrical mean expression and a statistically significant p-value (<0.05) based on analysis of DEseq2, were selected as the differentially expressed genes. To determine statistically enriched GO categories, the following GO analysis of those selected genes was conducted by an R package-Clusterprofiler with p<0.05. Based on Kyoto Encyclopedia of Genes and Genomes Database (http://www.genome.jp/kegg/), pathway analysis was employed to determine the significant pathway of those differential genes. mRNA-Seq and miRNA-Seq data are deposited in the Gene Expression Omnibus repository (GSE142517).
Quantitative Real-time RT–PCR. All the RNA was extracted by Trizol reagent (Invitrogen). Reverse transcription of miRNA to cDNA was implemented with miRNA-specific primers (Applied Biosystems) preceding the qPCR (TaqMan; Applied Biosystems). Noncoding small RNA control U6 (Applied Biosystems) was adopted served as an endogenous reference gene, with changes in expression calculated by the change in threshold (ΔΔCT) method. For mRNA, cDNA was prepared by reverse transcription (PrimeScript RT Reagent kit; Takara). The same amounts of cDNA were adopted for the following qPCR conducted with the SYBR PrimeScript reverse-transcription (RT)-PCR kit (Takara). Amplification was implemented through employing an ABI PRISM 7900 Real Time PCR System (Applied Biosystems). The amplification efficiency of these genes was the same as that for β-ACTIN or RPL13a, as indicated by the standard curves for amplification, allowing us to adopt the subsequent formula, fold difference = \(2^{-\Delta\Delta CT}\), where CT is the cycle threshold. Primer sequences are presented in Supplemental Table 3.

miRNA target prediction. Targetscan version 7.1 (http://www.targetscan.org/mmu_71/) was adopted to predict the miR-148a targets. The detailed descriptions of the complete computational protocol are available at the website and in reference (47).

Luciferase assay. For 3’UTR reporter assays, the 3’UTR of the MAFB mRNA containing WT or mutant miR-148a binding sites were subcloned into the psiCHECK2 vector (Promega). Then 1×10^5 HEK 293T cells per well was transfected with a mixture of 100ng 3’UTR luciferase reporter vector and agomir-miR-148a or agomir control using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. 24 hours after transfection, cells were lysed, and luciferase activity was measured on a luminometer (Promega) by using the Dual-Luciferase Reporter Assay System (Promega). The ratio of renilla luciferase activity to firefly luciferase activity was calculated for each well.

For promoter reporter assay, 200 bp upstream of the TSS of miR-148a with or without a mutant PU.1 binding sequence were subcloned into the pGL3-basic vector (Promega). Then 1×10^4 HeLa cells per well were transfected with a mixture of 100ng
pGL3 luciferase vector and 8ng pRL-TK Renilla vector with or without 100ng PU.1 expression plasmid or empty control plasmid. 24 hours after transfection, cells were lysed, and luciferase activity was measured on a luminometer (Promega) using the Dual-Luciferase Reporter Assay System. The ratio of firefly luciferase to renilla luciferase was calculated for each well.

**Chromatin Immunoprecipitation (ChIP) assays.** ChIP assays were conducted with a SimpleChIP® Plus Enzymatic Chromatin IP (Magnetic Beads) Kit (Cell Signaling Technology). According to the protocol, monocytes were fixed in 1% formaldehyde and then lysed for 10 minutes on ice. Chromatin was sheared by enzyme digestion and sonication. Then the cell lysate was immunoprecipitated overnight at 4°C with a monoclonal antibody anti-PU.1 (Cell Signaling Technology, 2258). Subsequently, protein G magnetic beads were added, and the obtained mixture was rotated for 2 hours at 4°C. After washing and elution, cross-links were reversed by heating at 65°C for 2 hours. The eluted DNA was purified, and the samples were analyzed by qPCR with input DNA (total chromatin) as an endogenous control. The promoter in CD11b and downstream of miR-148a TSS region were set as positive or negative control, respectively. ChIP-qPCR primer sequences are listed in Supplemental Table 3.

**Ago2 immunoprecipitation.** Ago2 immunoprecipitation was carried out according to the protocol provided in the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, 17-700). For the RNA-binding protein immunoprecipitation (RIP) lysate preparation, 2×10^7 human primary monocytes were collected by centrifugation at 1,500 revolutions per minute for 5 minutes and then lysed with RIP lysis buffer. For the magnetic bead/antibody preparation, purified antibodies (5µg) were incubated with beads for 30 minutes at room temperature, using anti-Ago2 antibody (Millipore, 03-110) as the antibody of interest and normal mouse IgG as negative controls, respectively. Supernatant from the RIP lysate was obtained by centrifugation at 14,000 rpm for 10 minutes, 100µL of which was incubated overnight at 4°C with bead-antibody complex, and 10µL served as 10% input for generating a standard curve or for qRT-PCR
comparison. Immunoprecipitated RNAs were purified and analyzed by quantitative qRT-PCR. RIP-qPCR primer sequences are listed in Supplemental Table 3.

**Immunoblots.** Cell lysates were prepared through lysing $2 \times 10^6$ monocytes or moDCs in radioimmune precipitation assay lysis and extraction buffer (Thermo Fisher Scientific), and then separated with 10% SDS-PAGE. The separated proteins were transferred to an Immobilon-P polyvinylidene difluoride membrane (Millipore, Billerica, MA), which was subsequently blocked with SuperBlock T20 PBS blocking buffer (Thermo Fisher Scientific, Pittsburgh, PA). Finally, the membrane was incubated with rabbit antibodies against MAFB, 1:1000 (Abcam, ab66506), PU.1, 1:1000 (Cell Signaling Technology, 2258) and β-ACTIN, 1:2000 (Cell Signaling Technology, 4967).

**Cytokine detection.** Mouse serum were collected at specific timepoint, and the protein levels of IL23, IL17, IL1β, IL6 and TNFα were determined with BD™ Cytometric Bead Array (CBA) Mouse/Rat Soluble Protein Master Buffer Kit and Flex Sets, according to the instruction manual provided by the manufacture. Briefly, the phycoerythrin (PE) conjugated detection antibodies are mixed with the specific capture beads, then incubated with recombinant protein standards or samples to generate sandwich complexes. Subsequently, the sandwich complexes of each sample are running on a flow cytometer. Standard curves for all analytes were generated through adopting recombinant protein standards to run one standard mixture. Finally, the sample results were analyzed by FCAP Array™ software (BD).

**Statistics.** Considering the clinical data, Nonparametric Mann-Whitney U test was employed to draw comparisons between patients and controls, and Spearman’s test was adopted to carry out the correlation analysis. For all other experiments, data were analyzed through using Graph Prism 7.0 software (GraphPad Software Inc.), and p values were determined by two-tailed unpaired t test or one-way ANOVA. * p < 0.05, ** p < 0.01, *** p < 0.001, n.s., not significant.
Study approval. Human sample acquisitions were approved by the Review Board of Renji Hospital of Shanghai Jiao Tong University School of Medicine and Huashan Hospital of Fudan University. All experiments were conducted in accordance with the declaration of Helsinki Principles. Informed consent was obtained for all procedures. All the animal experiments were performed with the use of protocols approved by the Institutional Animal Care and Use Committee at Renji Hospital of Shanghai Jiao Tong University School of Medicine.
Author contributions

N.S., H.Z. and Y.M. designed the project. Y.M. and J.L. performed the experiments, and Y.M., J.L., and H.Z. analyzed the data. Z.Y. and Z.Y. analyzed the bioinformatics data. G.L. established the mouse model. Y.Y., L.L. and J.D. collected the human samples. Q.S. and Z.L. participated in the transfection experiments. Y.M., J.L., H.Z., and N.S. prepared the manuscript.
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References


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**Figures and Figure legends**

**Figure 1. MiR-148a is indispensable for moDC differentiation.** (A, B) Monocytes isolated from BM of WT or miR-148a<sup>-/-</sup> mice were cultured with 50ng/mL of GM-CSF and 20ng/mL IL4 for 5 days. Flow cytometry analysis (A), morphology (B) and statistical data (C) of moDCs and macrophages are shown. (D) Flow cytometry and statistical data of apoptosis during monocyte differentiation at indicated time points were analyzed (n=3). Scale bars represent 10µm in (B). p values were determined by two-tailed unpaired t test Error bars represent SEM. (***p<0.001, n.s., not significant).
Figure 2. MAFB is a direct target of miR-148a. (A, B) Monocytes isolated from miR-148a<sup>−/−</sup> or WT BM were cultured with GM-CSF and IL4, or not. Gene expression were detected by mRNA sequencing. (A) Gene ontology (GO) analysis of differentially expressed genes. (B) Venn diagram of upregulated genes in miR-148a<sup>−/−</sup> monocytes on day 0 and 3 is shown. Upregulated genes with predicted miR-148a targets are listed. (n=3). (C) Predicted binding site of miR-148a in the 3′UTR of MAFB and the mutation of binding site. (D) Immunoblot analysis of MAFB expression in monocytes isolated from WT or miR-148a<sup>−/−</sup> BM. The target gene protein/β-ACTIN ratio in WT group was set as 1. (E) RIP assay. Human primary monocytes were transfected with control (NC) or Agomir-148a. QRT-PCR analysis of enriched MAFB mRNA levels after Ago2
immunoprecipitation. IgG was used as negative control. (n=3). (F) Dual luciferase reporter assays showing direct interaction of miR-148a with its cognate binding sites in the 3′UTR of MAFB. MAFB 3′UTR mutant refers to construct with mutated cognate binding sites for miR-148a. The renilla luciferase (Renilla) signal regulated by the target genes 3′UTR was normalized by the firefly luciferase (Firefly). The Renilla/Firefly ratio was set as 1 for the control psiCHECK2 plasmid. (n=3). (G) Knockdown of MAFB reduced the difference in the differentiation of moDCs between WT and miR-148a−/− monocytes. Monocytes derived from WT and miR-148a−/− mice were transfected with control (NC) or MAFB siRNA, and then cultured with GM-CSF and IL4 for indicated days. The differentiation of moDCs was analyzed. (n=3). Statistical analysis was performed with two-tailed unpaired t test or one-way ANOVA with Bonferroni’s post hoc test. Error bars represent SEM (*p<0.05, **p<0.01, ***p<0.001, n.s., not significant).
Figure 3. MiR-148a is involved in the inhibition of MAFB expression by PU.1. (A) Sequence alignment of the human and mouse miR-148a promoter (-strand) showing localization of predicted PU.1 binding site (blue). The transcriptional start sites are highlighted in red. Genomic locations for the human (GRCh38/hg18) and mouse (GRCm38/mm10) sequence intervals are provided above. (B) Taqman qRT-PCR analysis of miR-148a expression in control (NC) or PU.1 siRNA transfected human monocytes. U6 snRNA was used as endogenous control. (n=3). (C, D) Kinetic analysis of miR-148a, PU.1 and MAFB expression during moDC differentiation. (C) mRNA levels of PU.1 and MAFB were detected by qRT-PCR and normalized to the β-ACTIN RNA level. The mRNA/β-ACTIN ratio on day 0 was set as 1. (D) Immunoblot analysis. The protein/β-ACTIN ratio on day 0 was set as 1. (E) Monocytes isolated from WT and miR-148a−/− BM cells were transfected with control (NC) or siRNA specific to PU.1 (si-PU.1), and the protein levels of MAFB were detected. The MAFB/β-ACTIN ratio in WT+NC was set as 1. (F) Luciferase reporter assay in Hela cells co-transfected with the promoter of miR-148a (148-luc) or miR-148a promoter lacking the PU.1 binding site (mut-luc), and a vector overexpressing PU.1. The firefly luciferase signal was normalized by renilla luciferase. The Firefly/Renilla ratio was set as 1 for the no promoter empty plasmid (luc). (n=3). (G) Binding of PU.1 within the miR-148a promoter was analyzed using ChiP and qRT-PCR on human monocytes collected on day 0 or 3 during moDC differentiation. The ets site in the CD11b promoter was set as positive control, while the downstream of miR-148 TSS was as negative control (neg). Data are the mean percentage of input ± SEM. (n=3). p values compare
the indicated groups using a two-tailed unpaired *t* test or one-way ANOVA with Bonferroni’s post hoc test. Error bars represent SEM (*p<0.05, **p<0.01, ***p<0.001, n.s., not significant).
Figure 4. MiR-148a deficiency alleviates the development of psoriasis. WT and miR-148a−/− mice were treated with IMQ for 6 consecutive days. (A) Phenotypic presentation of skin lesions of representative mice on day 6. (B) Ear thickness. (C) PASI score. (D) H&E staining. (E) Dermal thickness. (F) Acanthosis. (n=5). (G) The relative mRNA expression of indicated cytokines in skin (upper) and the protein levels (lower) of these cytokines in serum on day 4 (n=5). (H) Flow cytometry analysis of the skin infiltration of moDCs (CD45+MHCIIfhiLy6C−CD64+CD11c−CD11b+), macrophages (CD45+MHCIiLy6C+CD64+CD11c+CD11b−), and IL17+ γδT cells (CD45+CD3−γδTCR−IL17A+).
(n=5). (I) Statistical data of the skin infiltration of moDCs, macrophages and IL17+ γδT cells (n=5). Scale bars represent 100μm in (D). p values were determined by a two-tailed unpaired t test. Error bars represent SEM (*p<0.05, **p<0.01, ***p<0.001, n.s., not significant).
Figure 5. MoDC intrinsic miR-148a contributes to inflammation. (A) Schematic diagram for IMQ application and depletion of monocytes plus neutrophils or neutrophils alone in WT or miR-148a−/− mice. Phenotypic presentation (B) and H&E staining (D) of skin lesions of representative mice on day 5 (n=3). Decrease in ear thickness (C, upper), PASI score (C, lower), dermal thickness (E, upper) and acanthosis (E, lower) on day 5 (n=3). (F) The relative mRNA expression of indicated cytokines in skin (n=3). (G) Statistical data of the skin infiltration of moDCs and IL17+ γδ T cells (n=3). Scale bars represent 100µm in (D). p values were determined by a two-tailed unpaired t test or by one-way ANOVA with Bonferroni’s post hoc test. Error bars represent SEM. (*p<0.05, **p<0.01, ***p<0.001, n.s., not significant).
Figure 6. Intradermal administration of antagonmir-148a ameliorates the pathological phenotype of IMQ-induced psoriasis-like mice. (A) Schematic diagram for IMQ application and intradermal administration of antagonmir-NC or antagonmir-148a. Phenotypic presentation (B) and
H&E staining (D) of skin lesions of representative mice on day 5 ($n=6$). Decrease in PASI score (C) and ear thickness (E) during the course of disease ($n=6$). (F) The relative mRNA expression of indicated cytokines in skin ($n=6$). Flow cytometry analysis (G) and statistical data (H) of the skin infiltration of moDCs and IL17$^+$ γδ T cells ($n=6$). Scale bars represent 100µm in (D). p values were determined by a two-tailed unpaired $t$ test or one-way ANOVA with Bonferroni’s post hoc test. Error bars represent SEM (*$p<0.05$, **$p<0.01$, ***$p<0.001$, n.s., not significant).
Figure 7. The expression of miR-148a is elevated in psoriatic monocytes. (A) Pro-inflammatory cytokine gene expression in the PBMCs derived from psoriasis patients (n=34) and healthy controls (n=51). (B) Expression of miR-148a in peripheral monocytes isolated from psoriasis patients (n=34) and healthy controls (n=51). (C) The correlation analysis between the miR-148a level in psoriatic monocytes and the mRNA level of IL23a and IL17a in psoriatic PBMCs (n=34). (D, E) Expression of MAFB (D) and PU.1 (E) in peripheral monocytes isolated from psoriasis patients (n=20) and healthy controls (n=23), and the correlation analysis between the miR-148a and MAFB (D) or PU.1 (E) level in psoriatic patients (n=20). p values compare the indicated groups using Nonparametric Mann-Whitney U test or Spearman’s test. Error bars represent SEM (*p<0.05, **p<0.01, ***p<0.001, n.s., not significant).
Figure 8. Regulation model. MiR-148a, which could be transcriptionally activated by PU.1, promotes moDC differentiation through directly targeting MAFB, therefore causing the immune imbalance and inflammatory response.