CCR7 Chemokine Receptor-Inducible Inc-Dpf3 Restrains Dendritic Cell Migration by Inhibiting HIF-1α-Mediated Glycolysis

Graphical Abstract

Highlights

- Inc-Dpf3 feedback restrains CCR7-induced DC migration and inflammatory response
- CCR7 stimulation upregulates Inc-Dpf3 via m6A demethylation to prevent its degradation
- Inc-Dpf3 suppresses DC migration via inhibiting HIF1α-dependent glycolysis
- Inc-Dpf3 directly binds to HIF1α and inhibits HIF1α activity via an HRE motif

Authors
Juan Liu, Xiaomin Zhang, Kun Chen, ..., Ha Zhu, Zhiqing Li, Xuetao Cao

Correspondence
juanliu@immunol.org (J.L.), caoxt@immunol.org (X.C.)

In Brief
CCR7-mediated DC migration needs to be suppressed in a timely manner to avoid aberrant inflammatory responses. Liu and colleagues demonstrate that feedback of a long non-coding RNA Inc-Dpf3 restrains CCR7-mediated DC migration via directly inhibiting HIF-1α-dependent glycolysis.
**INTRODUCTION**

Epigenetic pathways have been increasingly shown to be important in the regulation of biological processes. In particular, long non-coding RNAs (lncRNAs) act in a variety of ways to regulate immune responses in a lineage- or signal-dependent manner (Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNAs are identified to modulate inflammatory response and antiviral immunity, such as lincRNA-Cox2 (Carpenter et al., 2013), lincRNA-EPS (Atianand Rinn and Chang, 2012). Several lncRNA...
Figure 1. Inc-Dpf3 Negatively Regulates CCR7-Dependent Dendritic Cell Migration

(A) Heatmaps of the differentially expressed lncRNAs in mDCs stimulated with CCR7 ligand CCL19 and CCL21 (CCR7L) for 12 h or left unstimulated (Med) (fold change > 2, p < 0.05).

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its degradation in DCs. Subsequently, the upregulated Inc-Dpf3 feedback interacted with transcription factor HIF-1α (hypoxia-inducible factor 1-alpha) and consequently inhibited HIF-1α-depensent glycolysis and migratory capacity of DC. Our study sheds additional light on the molecular mechanisms for timely termination of DC migration and attenuating their immune-activating function at the late stage of an inflammatory immune response and provides additional insights into the balanced network of DC-based immune responses.

RESULTS

Inc-Dpf3 Suppresses CCR7-Mediated DC Migration

We previously identified the STAT3-binding Inc-DC as a critical controller of DC maturation (Wang et al., 2014a). However, the function of IncRNAs in regulation of DC migration remains unknown. It was shown that a rapid but transient migration of respiratory DCs from lungs to the draining lymph nodes (dLNs) occurs as early as 6 h and quickly slows down after 18 h post pulmonary virus infection (Legge and Braciale, 2003). A similar pattern of transient increase in DC migration from skin to dLNs was induced within 24 h by skin irritation (Tomura et al., 2014). Therefore, we suspected CCR7 stimulation might induce intracellular alterations after the peak of DC migration to dLNs (approximately 18–24 h post in vivo infection or irritation). Which functions to feedback-prevent excessive DC accumulation. So, we prepared LPS-stimulated bone marrow-derived DCs (mature DCs, mDCs) and then stimulated mDCs with or without CCR7 ligands CCL19 and CCL21 for 12 h in vitro. Through IncRNA profiling, we detected a total of 34 IncRNAs that were differentially expressed (18 upregulated and 26 downregulated, by fold change > 2) in CCR7-stimulated mDCs (Figure 1A; Table S1). qRT-PCR analysis of the 18 upregulated IncRNAs confirmed that 6 of them were upregulated significantly after CCR7 stimulation (Figure 1B). By performing RNA interference (RNAi)-mediated functional screening of these 6 IncRNAs, we found that CCR7-mediated mDC migration in vitro was potently increased by silencing of an intronic IncRNA (noncode database ID: m274819; GenBank: AK140952.1) (Figure 1C), which was located within intron 1 of the mouse Dpf3 gene and is named Inc-Dpf3 hereafter.

Rapid amplification of cDNA ends (RACE) identified the Inc-Dpf3 sequence with 4,076 nucleotides (nt) (Figure S1A). Like most identified IncRNAs, Inc-Dpf3 had a S’ cap structure and polyA tail but had no protein-coding capacity (Figure S1B). It had a moderate expression, about 130 ± 15 transcript copies per cell in DCs (Figure S1C). It was expressed in multiple organs (Figure S1D) and was expressed highly in splenic CD11c+ DCs compared to those of splenic B cells, T cells, or NK cells (Figure S1E). We confirmed that silencing of Inc-Dpf3 with different sets of siencers potently increased CCR7-mediated mDC migration (Figures 1D and S1F). However, Inc-Dpf3 silencing had no effect in expression of CCR7 and I-Aβ or apoptotic responses in DCs (Figures S1G and S1H). These data indicate that Inc-Dpf3 is involved in suppression of DC migration in vitro.

DC-Specific Inc-Dpf3 Deficiency Selectively Promotes CCR7-Mediated DC Migration

To further explore the biological role of Inc-Dpf3, we generated Inc-Dpf3fl/fltgax-cemice with specific deletion of Inc-Dpf3 in CD11c+ DCs from mice through homologous recombination (Figures S2A and S2B). We confirmed the deficiency of Inc-Dpf3 expression in CD11c+ DCs from Inc-Dpf3fl/fltgax-cre+ mice (Figure S2C). The frequency of major immune cell subsets and the expression of CCR7 on CD11c+ splenic DCs were intact in Inc-Dpf3fl/fltgax-cre+ mice (Figure S2D). In addition, BMDCs (bone-marrow-derived dendritic cells) generated from Inc-Dpf3fl/fltgax-cre+ mice had normal phenotypic maturation, proinflammatory cytokine production, and ability to stimulate T cell proliferation and activation (Figures S2E–S2H). Therefore, DC-specific deficiency of Inc-Dpf3 does not affect the differentiation of major immune cell subsets or the maturation and T-cell-stimulating capacity of DCs.

We next determined whether Inc-Dpf3 deficiency affected CCR7-triggered DC migration. We found that both immature and mature DCs from Inc-Dpf3fl/fltgax-cre+ mice showed increased migration after 12 h of CCR7 stimulation compared to DCs from their littermates (Figures 1E and S3A). In line with this, upregulation of Inc-Dpf3 by CCR7 stimulation was independent of LPS stimulation (Figure S3B). Adenovirus-mediated overexpression of Inc-Dpf3 reduced CCR7-triggered DC migration after 12 h (Figure 1F). However, the migration speed of DCs during the first 4 h after CCR7 stimulation was not altered in Inc-Dpf3fl/fltgax-cre+ DCs (Figure S3C), indicating that Inc-Dpf3 does not affect DC migration at the early stage after CCR7 stimulation. In addition, Inc-Dpf3 expression was continuously upregulated after 12 h of CCR7 stimulation in vitro (Figure S3D). These data suggest a selective role for Inc-Dpf3 in feedback-inhibiting late-stage DC migration triggered by CCR7 in vitro.

We next investigated the function of Inc-Dpf3 in CCR7-mediated DC migration in vivo. Inc-Dpf3 silencing mDCs displayed increased migration toward dLNs at the late phase (24–48 h) post injection compared to control mDCs (Figures 1G and 1H). Consistently, mDCs from Inc-Dpf3fl/fltgax-cre+ mice also displayed increased migration toward dLNs compared to control mDCs at 24–48 h post injection (Figures 1I and S3E). After skin painting with FITC (fluorescein isothiocyanate), the proportions

(B) qRT-PCR analysis of gene expression of selected IncRNAs in mDC.
(C) CCR7-triggered migration of mDCs transfected with siencers specific for 6 selected IncRNAs or a control siencer (si-NC), analyzed in a Transwell chamber.
(D) CCR7-triggered migration of mDCs transfected with three different sets of siencers of Inc-Dpf3 (si-Inc-Dpf3 #1, #2, and #3).
(E) CCR7-triggered migration of imDCs and mDCs from Inc-Dpf3fl/fltgax-cre+ and Inc-Dpf3fl/fltgax-cre− mice.
(F) CCR7-triggered migration of mDCs transfected with adenovirus overexpressing Inc-Dpf3 (Ad-Inc-Dpf3) or control adenovirus (Ad-NC).
(G) In vivo migration of carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled mDCs transfected with si-NC or si-Inc-Dpf3 #1, #2, and #3 (G) or si-Inc-Dpf3 #1 (H) or from Inc-Dpf3fl/fltgax-cre+ and Inc-Dpf3fl/fltgax-cre− mice (I).
(J–L) Expression of FITC within CD11c+ DC (J), distinct DC subsets (K), or migratory DC subsets (L) in dLNs of Inc-Dpf3fl/fltgax-cre+ and Inc-Dpf3fl/fltgax-cre− mice at 48 h following FITC skin painting.

Data are from one experiment (A) or one representative of three independent experiments and are shown as one typical result (I–L) or mean ± SD of indicated determinants (B–H). *p < 0.05, **p < 0.01, NS, not significant (unpaired two-tailed Student’s t test). See also Figures S1 and S2 and Table S1.
Figure 2. Inc-Dpf3 Restrains Inflammatory Injuries during CHS Responses

(A and B) Net changes in ear thickness at indicated time (A) and ear histology at day 3 post challenge (B) in Inc-Dpf3<sup>fl/fl</sup>Itgax-cre<sup>+</sup> and Inc-Dpf3<sup>fl/fl</sup>Itgax-cre<sup>/-</sup> mice after CHS induction. Scale bars, 100 μm in (B).

(C and D) 5 days after DNFB sensitization, dLNs cells were re-stimulated with DNBS ex vivo for 3 days and assessed with the proportion of Th1 and Th17 cells (C) and the concentrations of IFN-γ and IL-17 in cell culture supernatants (D).

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of FITC+ DCs in dLNs were increased in Inc-Dpf3fl/flItgax-cre− mice at 48 h following FITC painting (Figure 1J and S3F). Thus, Inc-Dpf3 inhibits late-stage DC migration toward dLNs in vivo.

The total CD11c+ DCs in dLNs can be divided into four major subsets, including monocyte-derived DCs (moDCs, CD11cintCD40lo), migratory cDCs (CD11cintCD40hi), resident cDCs (CD11chiCD40lo), and Langerhans cells (LCs, CD11chiCD40hi) (Ruedl et al., 2000; Guilliams et al., 2016). We found that only the CCR7-dependent migratory DC subsets (migratory cDCs and LCs), but not the CCR7-independent migratory DC subset (moDCs) (Nakano et al., 2009), showed increased FITC expression in Inc-Dpf3fl/flItgax-cre+mice (Figures 1K and S3G). The migratory cDCs can be further divided into XCR1+ migratory cDC1 and XCR1− migratory cDC2, and these two subsets both had enhanced FITC expression in Inc-Dpf3fl/flItgax-cre+mice (Figures 1L and S3G). These data further confirmed that Inc-Dpf3 selectively inhibits DC migration in a CCR7-dependent manner.

In addition, Inc-Dpf3 was induced in FITC+ DCs after 48 h of DC migration and returned to steady-state expression after 96 h, possibly due to cell death of migratory DCs (Liu et al., 2007) (Figure S3H). Moreover, the FITC+ migratory DCs had significantly higher expression of Inc-Dpf3 expression than the FITC− resident DCs in the dLNs (Figure S3I). Together, these data indicated that Inc-Dpf3 is upregulated during CCR7-mediated DC migration and subsequently feedback-inhibits DC migration both in vitro and in vivo.

DC-Specific Inc-Dpf3-Deficient Mice Develop More Severe Inflammation

We next explored the physiological role of Inc-Dpf3 in controlling DC-dependent inflammatory response in vivo. Mouse contact hypersensitivity (CHS) with 2,4-dinitro-fluorobenzene (DNFB) as an antigen is a well-established model of human-contact dermatitis dependent on CCR7-mediated DC trafficking toward dLNs. We found that Inc-Dpf3fl/flItgax-cre+mice developed more severe inflammation after CHS induction, as indicated by increased degree of ear swelling, more severe tissue injury, and inflammatory infiltration (Figures 2A, 2B, S3J, and S3K). Further evidence of inflammation of these mice was found in the expanded T helper 1 (Th1) and Th17 cell populations and higher concentrations of interferon (IFN)−γ and interleukin (IL)-17 in dLNs after DNBS re-stimulation ex vivo (Figures 2C and 2D) as compared to their littermates. In addition, mice that had received DNBS-loaded Inc-Dpf3-silencing mDCs developed a much stronger CHS response after DNFB challenge than mice without injection with DNBS-loaded control mDCs (Figures 2E–2G). Thus, Inc-Dpf3 in DCs attenuates adaptive responses and inflammatory injuries.

Because CCR7-mediated DC migration toward dLNs under steady conditions contributes to peripheral tolerance, we wondered whether Inc-Dpf3 was involved in steady-state DC migration. The migratory DCs in dLNs can generally be distinguished from resident DCs on the basis of CCR7+ expression (Baratin et al., 2015). We found that the migration of cDC1 and cDC2 toward dLNs was enhanced in Inc-Dpf3fl/flItgax-cre+mice compared to their littermates only after DNFB stimulation but not under steady-state conditions (Figure 2H). The proportion of regulatory T cells was normal in Inc-Dpf3fl/flItgax-cre+mice (Figure S3L). Therefore, Inc-Dpf3 does not affect either steady-state DC migration or generation of peripheral tolerance. However, the migratory cDC1 (XCR1+CCR7+) had higher expression of Inc-Dpf3 than non-migratory cDC1 (XCR1+CCR7−) in the dLNs under both homeostatic and inflammatory conditions (Figure 2I); therefore, the expression of Inc-Dpf3 is upregulated during CCR7-mediated migration independent of inflammatory triggers. This is consistent with the in vitro data showing that CCR7 stimulation induced upregulation of Inc-Dpf3 in both immature DCs and LPS-induced mature DCs (Figure S3B). Together, these results show that although Inc-Dpf3 is upregulated in migratory DCs under both steady-state and inflammatory conditions, it selectively attenuates CCR7-mediated DC migration under inflammatory conditions.

CCR7 Stimulation Upregulates Inc-Dpf3 Expression by Removing m6A Modification to Prevent Its Degradation

As the dynamic expression of Inc-Dpf3 is closely related to its mode of regulatory function, we wondered how Inc-Dpf3 is regulated by CCR7 stimulation. We first sought to determine whether CCR7 stimulation induced Inc-Dpf3 gene expression on a transcriptional level. With chromatin immunoprecipitation (ChIP) experiments, we found that the abundance of chromatin activation mark H3K4me3 and the occupancy of DNA polymerase II at the promoter regions of Inc-Dpf3 were not affected by CCR7 stimulation (Figure S4A). In addition, Inc-Dpf3 induction by CCR7 stimulation was not impaired by transcription inhibition, further confirming that CCR7 did not increase Inc-Dpf3 expression via transcription mechanisms (Figure S4B). Next, we investigated whether CCR7 stimulation affected the subcellular location of Inc-Dpf3. We found that Inc-Dpf3 was localized mainly in the cytosol with a small portion located in the nucleus, which was confirmed using single-molecule RNA fluorescent in situ hybridization (FISH) in mDCs (Figures 3A and 3B). However, CCR7 stimulation did not obviously affect the subcellular distribution of Inc-Dpf3 (Figure 3A).

Consequently, we wondered whether or not CCR7 stimulation increased Inc-Dpf3 expression via post-transcriptional regulation of RNA stability, m6A is the first identified and most abundant internal modification of RNA that broadly affects RNA metabolism and stability (Roundtree et al., 2017; Gilbert et al., 2016; Dominissini et al., 2012). As such, we determined whether the
Figure 3. CCR7 Stimulation Upregulates Inc-Dpf3 Expression by Removing m^6^A Modification

(A) Relative expression of RNA purified from nuclear and cytosolic compartments in mDC.

(B) Single-molecule RNA FISH detecting endogenous lnc-Dpf3 molecules (green) in mDC. DNA (blue) was stained with DAPI. Scale bars, 10 μm.

(C) Left: Me-RIP assay of m^6^A enrichments at distinct fragments of lnc-Dpf3 in mDC. Right: quantification result of m^6^A enrichment at the 22nd fragment (3,766–3,903 nt) of lnc-Dpf3.

(D) RIP assay of Ythdf1 and Ythdf2 enrichment at the 22nd fragment of lnc-Dpf3 in mDC.

(E) Immunoblot analysis of Ythdf1 and Ythdf2 protein expression in mDC.

(F) lnc-Dpf3 expression in mDCs transfected with siRNA specific for Ythdf1 or Ythdf2 (si-Ythdf1, si-Ythdf2) or si-NC.

(G) Half-life of lnc-Dpf3 RNA in HEK293T cells transfected with plasmids overexpressing WT lnc-Dpf3 or its m^6^A site mutations (Δm^6^A-1, Δm^6^A-2) after transcription inhibition with actinomycin D (Act D).

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dynamic expression of Inc-Dpf3 in DCs was regulated in an m6A-relevant manner. We tested the existence of m6A modification on Inc-Dpf3 by m6A RNA immunoprecipitation (Me-RIP) followed by qPCR with 23 pairs of primers (designed for every 180 nts of the full RNA sequence). We detected a significant enrichment of m6A at the 22nd fragment (3,766–3,903 nt) of Inc-Dpf3 in resting mDCs, and the m6A peak was markedly reduced by CCR7 stimulation (Figure 3C). RIP assay showed that the m6A peak of Inc-Dpf3 was recognized by m6A reader Ythdf2 (YTH N6-methyladenosine RNA binding protein 2), but not Ythdf1, and the recognition of Inc-Dpf3 by Ythdf2 was also downregulated by CCR7 stimulation (Figure 3D). The expression of Ythdf1 or Ythdf2 themselves was not affected by CCR7 stimulation (Figure 3E).

Moreover, silencing of Ythdf2 with siRNA could upregulate Inc-Dpf3 expression (Figure 3F). This is consistent with the previous finding that Ythdf2 binding could accelerate degradation of methylated transcripts (Wang et al., 2014b, 2015; Du et al., 2016).

Inc-Dpf3 contains a total of 14 m6A motifs (GGAC), and two of them locate within the 22nd fragment at 3,812–3,815 nt and 3,852–3,855 nt. Consequently, we generated plasmids overexpressing wild-type (WT) Inc-Dpf3 or the two m6A motif mutations of Inc-Dpf3 with the GGAC site mutated into GGGC (Δm6A-1 mutation at 3,812–3,815 nt, Δm6A-2 mutation at 3,852–3,855 nt). Overexpression of Δm6A-1 showed that Δm6A-2 itself led to prolonged lifetime of Inc-Dpf3 compared to WT Inc-Dpf3, indicating that m6A modification at 3,812–3,815 nt and 3,852–3,855 nt of Inc-Dpf3 contributes to the decay of Inc-Dpf3 RNA (Figure 3G).

To clarify whether these two m6A motifs function in a synergistic manner, we generated a Inc-Dpf3 mutation of both motifs (Δm6A-1+2). Me-RIP assay confirmed that the high abundance of m6A modification in WT Inc-Dpf3 was reduced in Inc-Dpf3 Δm6A-1 and Δm6A-2 mutations and was most obviously reduced in the Δm6A-1+2 mutation (Figure 3H). Consistently, the expression of Inc-Dpf3 was higher in Δm6A-1 or Δm6A-2 mutations and was most obviously increased in the Δm6A-1+2 mutation (Figure 3I). Therefore, m6A modifications at 3,812–3,815 nt and 3,852–3,855 nt of Inc-Dpf3 play synergistic roles in mediating RNA degradation of Inc-Dpf3. Collectively, Inc-Dpf3 bears m6A modifications at 3,812–3,815 nt and 3,852–3,855 nt, which are recognized and targeted by reader Ythdf2. CCR7 stimulation upregulates Inc-Dpf3 expression by mediating m6A demethylation and relieving m6A-dependent RNA degradation of Inc-Dpf3.

**CCR7-Inducible Inc-Dpf3 Suppresses DC Migration via Targeting HIF-1α**

IncRNAs have been reported to have cis-regulatory effects on nearby genes. We found that Inc-Dpf3 silencing impaired expression of its cis gene Dpf3 but not two other nearby genes Rgs6 and Dcaf3 (Figures S4C and S4D). However, silencing of Dpf3 did not affect DC migration (Figure S4E). Also, overexpression of Dpf3 did not reverse the enhanced DC migration in Inc-Dpf3 Δm6A-1+2 CCR7+/- mice (Figures S4F and S4G). These data exclude a role of Dpf3 in the function of Inc-Dpf3 in regulating DC migration.

Next, we wondered whether Inc-Dpf3 affected the major signaling implicated in the innate activation of DCs. We found that Inc-Dpf3 silencing did not affect the rapid phosphorylation of Akt (protein kinase B), mTOR (mechanistic target of rapamycin), or ERKs (extracellular signal-regulated kinases) induced by CCR7 in mDCs (Figure S4H). Notably, inhibition of Akt with AKT-IV or of its canonical activator PI3K (phosphoinositide 3-kinase) with wortmannin or of its downstream transcriptional factor HIF-1α with 2-Methoxyestradiol (2-ME) significantly blocked CCR7-dependent DC migration and reversed the enhanced DC migration in Inc-Dpf3 silencing DCs (Figure S4I). Thus, the silencing via PI3K, Akt, and HIF-1α might be involved in the function of Inc-Dpf3 in regulating CCR7-mediated DC migration.

HIF-1α is a center transcription factor of cell responses to hypoxic stress and plays essential roles in controlling various aspects of innate and adaptive immune responses (Cramer et al., 2003; Dang et al., 2011; Doedens et al., 2013). Considering the hypoxic microenvironments of infected tissues and lymph nodes, we suspected that Inc-Dpf3 might regulate CCR7-mediated DC migration via modulating HIF-1α activation. We found that DC-specific HIF-1α-deficient mice (Hif1α<sup>Cre<sup>ko</sup>) exhibited impaired DC migration in vitro (Figures S4J and S4K) and in vivo (Figures S4L and S4M) compared to their littermates. Thus, HIF-1α is required for CCR7-mediated DC migration.

Notably, Inc-Dpf3 silencing or deficiency in mDCs increased nuclear translocation of HIF-1α induced by CCR7 stimulation without affecting the abundance of total intercellular HIF-1α (Figures S4A–4D, S4H, and S5A). RNA-seq analysis confirmed that a series of HIF-1α-targeted glycolytic genes, such as Hk1 (hexokinase 1), Ldha (lactate dehydrogenase A), and Slc2a1 (SLC2A1 solute carrier family 2 member 1), but not other HIF-1α-targeted genes such as Vegfa (vascular endothelial growth factor A) and Pgk1 (phosphoglycerate kinase 1), were upregulated in Inc-Dpf3 silencing DCs compared to control DCs after CCR7 stimulation (Figure 4E; Table S2). qRT-PCR analysis confirmed that CCR7-induced glycolytic gene Ldha was obviously increased by Inc-Dpf3 silencing or deficiency in mDCs (Figures 4F, S5B, and S5C). Conversely, adenovirus-mediated overexpression of Inc-Dpf3 inhibited Ldha mRNA expression in CCR7-stimulated mDCs (Figure S5D). The increased expression of Ldha in Inc-Dpf3 silencing DCs at the protein level was confirmed by immunoblotting (Figure S5E). In addition, Inc-Dpf3 silencing or deficiency in mDCs increased HIF-1α DNA binding activity and accumulation at the promoter region of the Ldha gene in mDCs, as determined by electrophoretic mobility shift assay (EMSA) (Figures 4G and S5F) and ChIP analysis (Figures 4H and S5G), respectively. Moreover, inhibition of Akt or HIF-1α could reverse the elevated Ldha gene expression in

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(1) Me-RIP assay of m6A enrichments in Inc-Dpf3 in HEK293T cells transfected with plasmids overexpressing WT Inc-Dpf3 or its m6A site mutations (Δm6A-1, Δm6A-2, Δm6A-1+2).

(2) Inc-Dpf3 expression in HEK293T cell transfected as in (1).

Data are from one representative of three independent experiments and are shown as one typical result (B and left panels of C and E) or mean ± SD of indicated determinants (A and right panels of C, D, and F–I). *p < 0.05, **p < 0.01, NS, not significant (unpaired two-tailed Student’s t test). See also Figure S4.
Figure 4. Inc-Dpf3 Suppresses DC Migration via Inhibiting HIF-1α-Induced Ldha Transcription

(A) Confocal analysis of HIF-1α nuclear translocation in mDCs from Inc-Dpf3fl/fl Itgax-cre+ and Inc-Dpf3fl/fl Itgax-cre/C0 mice. DNA (blue) was stained with DAPI. Scale bars, 10 μm.

(B) Protein expression in whole-cell lysates and nuclear and cytosolic extracts of mDCs transfected with si-Inc-Dpf3 or si-NC.

(C and D) Protein expression in nuclear extracts of mDCs from Inc-Dpf3fl/fl Itgax-cre+ and Inc-Dpf3fl/fl Itgax-cre/C0 mice stimulated with CCR7L for indicated time (C) or at different concentrations (D).

(E) RNA-seq analysis of HIF-1α-regulated genes in mDCs transfected as in (B).

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Inc-Dpf3-silencing DCs (Figure S5H). These data indicate that Inc-Dpf3 attenuates CCR7-induced HIF-1α activity and the expression of glycolytic gene Ldhα.

We then investigated whether or not Inc-Dpf3 functions via HIF-1α. As expected, mDCs from Hif1a−/− mice had reduced mRNA expression of targeted genes Ldhα and Pdk1 after CCR7 stimulation compared to those from their littermates (Figures S5I and S5J). Importantly, HIF-1α deficiency could block the effect of Inc-Dpf3 silencing or overexpression in increasing or decreasing DC migration (Figure 4I), and Hif1α silencing by siRNA could abrogate the effect of Inc-Dpf3 silencing or deficiency in increasing DC migration (Figure S5K).

Conversely, hypoxia could promote CCR7-mediated DC migration and rescue the impaired DC migration in Inc-Dpf3-overexpressed DCs (Figure 4J). Collectively, these data indicate that Inc-Dpf3 suppresses CCR7-mediated DC migration via targeting HIF-1α.

**Inc-Dpf3 Suppresses DC Migration via Inhibiting HIF-1α-Dependent Glycolysis**

It has been shown that TLR signals trigger rapid glycolytic reprogramming in DCs for their anabolic demands (Everts et al., 2014); however, the metabolic basis for CCR7-dependent migration remains poorly understood. Consistent with the increases in glycolytic genes as detected in both RT-PCR and RNA-seq, unbiased metabolomic profiling in mDCs revealed selective CCR7-induced accumulation of metabolic intermediates associated with glucose metabolism, such as glucose 1-phosphate, glucose 6-phosphate, and fructose 6-phosphate, at 12 h after CCR7 stimulation (Figure 5A; Table S3). Metabolic pathway enrichment analysis (MPEA) indicated that CCR7 stimulation induces changes in the key metabolic pathways closely associated with glycolysis, such as starch and sucrose metabolism (Figure S5B).

We next analyzed the rate of extracellular acidification (ECAR) and oxygen consumption (OCR) as a measure of lactate production and mitochondrial capacity, respectively, in mDCs after CCR7 stimulation. We observed a rapid and persistent increase in ECAR of DCs after CCR7 stimulation (Figure 5Q). Although Akt inhibition could block the increased glycolytic response at both early (1 h after CCR7 stimulation) and late (12 h after) stages, HIF-1α inhibition could selectively inhibit the glycolytic burst at the late stage, which indicates that Akt activation is required for the entire process of glycolysis, but HIF-1α is particularly required for the late glycolysis induced by CCR7 (Figure 5D). The ECAR/OCR ratio as a measure of metabolic shift toward aerobic glycolysis was continuously increased by CCR7 stimulation and was higher in Inc-Dpf3-silencing DCs compared to control DCs after 12 h (Figure 5E).

CCR7-induced glycolysis was further confirmed by the increases in glucose uptake, lactate production, and the ratio of nicotinamide adenine dinucleotide (NAD+) to its reduced form (NADH), which was elevated by Inc-Dpf3 silencing or deficiency in mDCs (Figures 5F and S6A–S6C). Conversely, overexpression of Inc-Dpf3 or DC-specific HIF-1α deficiency could inhibit lactate production in CCR7-stimulated mDCs (Figures 5G and S6D). The extensive lactate production in Inc-Dpf3-silencing DCs could be reversed by inhibition of LDHA with oxamate (Figure S6E). Importantly, treatment of DCs with glycolytic inhibitor 2-deoxyglucose (2-DG) could inhibit CCR7-mediated DC migration and abrogate the effect of Inc-Dpf3 silencing or deficiency in increasing DC migration, both in vitro (Figures 5H and S6F) and in vivo (Figures 5I and S6G). Notably, inhibition of glycolysis could inhibit both the early stage and late stage of DC migration to dLNs in vivo, indicating that glycolysis is required for the entire process of CCR7-dependent DC migration (Figure S6H). Therefore, Inc-Dpf3 negatively controls the late stage of DC migration by suppressing glycolysis.

Glycolysis is able to fuel mitochondrial respiration by supplying carbon sources. So, we investigated the changes in mitochondrial fitness in mDCs. CCR7 stimulation led to increased mitochondrial activity in mDCs, as indicated by upregulation in OCR, spare respiratory capacity (SRC), and mitochondrial membrane potential, and Inc-Dpf3 silencing reduced mitochondrial activity in CCR7-stimulated DCs (Figures S7A and S7B). The abundance of mitochondria was decreased by CCR7 stimulation, which was not affected by Inc-Dpf3 (Figure S7C). CCR7-induced increase in the SRC was prevented by 2-DG or 2-ME, which indicated that the increase in mitochondrial activity was mediated by the enhanced glycolytic flux (Figure S7D). Taken together, these data indicate that CCR7 stimulation induces HIF-1α-dependent glycolytic responses and concomitant changes in mitochondrial respiration in DCs, and Inc-Dpf3 restrains CCR7-mediated DC migration by suppressing HIF-1α-dependent metabolic reprogramming toward aerobic glycolysis.

**Inc-Dpf3 Directly Inhibits HIF-1α via an HRE Motif to Suppress DC Glycolysis**

Because the mRNA expression of Hif1α itself was not affected by Inc-Dpf3 (Figures 4F and S5B–S5D), we suspected that Inc-Dpf3 might physically associate with HIF-1α to regulate its activation through post-translational mechanisms. The physical interaction between Inc-Dpf3 and HIF-1α was confirmed by RNA immunoprecipitation (RIP) assay (Figure 6A), FISH assay (Figure 6B), and proximity ligation assay (PLA) (Figure 6C), and this interaction was increased by CCR7 stimulation (Figure 6A). We next used the hypoxia response element (HRE)-driven luciferase reporter to measure HIF-1α activity in HEK293T cells, a cell line which had an expression of CCR7 proteins similar to that of mDCs (Figure 6D). CCR7 stimulation enhanced HIF-1α activity both under normoxia and hypoxia conditions in a manner
Figure 5. Inc-Dpf3 Suppresses DC Migration via Inhibiting HIF-1α-Dependent Glycolysis

(A and B) Unbiased metabolite profiling of mDC. Heatmaps show the differentially expressed metabolite (p < 0.05) (A). Metabolic pathway enrichment analysis shows the five pathways with the most significant change (in ranked order, i–v) (B). Two independent samples (#1 and #2) and their respective technical replicates (#1R and #2R) were analyzed for each group.

(C and D) ECAR of mDCs stimulated with CCR7L for indicated time (C) or pretreated with inhibitor for 1h and then stimulated with CCR7L for indicated time (D).

(E) ECAR/OCR in mDCs transfected with si-lnc-Dpf3 or si-NC.

(F) Glucose uptake, lactate production, and NAD+ versus NADH ratio in mDCs from lnc-Dpf3 fl/fl Itgax-cre+ and lnc-Dpf3 fl/fl Itgax-cre/C0 mice.

(G) Lactate production in mDCs transfected with adenovirus overexpressing lnc-Dpf3 (Ad-lnc-Dpf3) or control adenovirus (Ad-NC).

(H) CCR7-triggered migration of mDCs from lnc-Dpf3 fl/fl Itgax-cre+ and lnc-Dpf3 fl/fl Itgax-cre/C0 mice treated as indicated.

(I) In vivo migration of CFSE-labeled mDCs treated as indicated.

Data are from one experiment (A and B) or one representative of three independent experiments and are shown as one typical result (I) or mean ± SD of indicated determinants (C–H). *p < 0.05, **p < 0.01, NS, not significant (unpaired two-tailed Student’s t test). See also Figure S6 and Table S3.
Figure 6. Inc-Dpf3 Interacts with the N and C Termini of HIF-1α via Its 3,000–4,076 nt Region

(A) RIP assay of HIF-1α or p-p65 enrichment at Inc-Dpf3 in mDC.

(B) RNA FISH assay of Inc-Dpf3 followed by immunofluorescence detection of HIF-1α in mDC. Scale bars, 10 μm.

(C) rISH-PLA (RNA in situ hybridization proximity ligation assay) of interaction between Inc-Dpf3 and HIF-1α fragments in HEK293T cells transfected with indicated plasmids. Scale bars, 50 μm.

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dependent on Akt (Figure 6D). Overexpression of Inc-Dpf3, but not its antisense control, inhibited HIF-1α activation (Figure 6E). We then divided Inc-Dpf3 into four fragments to map the HIF-1α-binding region. Only the F4 fragment (3000–4076 nt), but not the other three fragments (F1, F2, and F3), had an activity similar to that of full-length Inc-Dpf3 in inhibiting HIF-1α activity (Figure 6F). RNA pull-down assay with biotin-labeled Inc-Dpf3 and its four fragments also confirmed that both full-length and F4 fragments of Inc-Dpf3 directly bound to HIF-1α (Figures 6G and 6H). These data identify HIF-1α as a binding partner for the F4 fragment of Inc-Dpf3.

To determine the region in HIF-1α that binds to Inc-Dpf3, we divided HIF-1α into four fragments, termed as N-terminal domain 1 (NT1, encompassing basic helix-loop-helix/Per-Arm-Sim, bHLH/PAS-A domain, 1–200 aa), N-terminal domain 2 (NT2, encompassing PAS-B domain, 201–400 aa), oxygen-dependent degradation domain (ODDD, 401–600 aa), and C-terminal domain (CT, 600–826 aa) (Lim et al., 2010). RIP assay confirmed that both the NT1 and CT domains of HIF-1α could bind to Inc-Dpf3, whereas the NT2 and ODDD domains are dispensable for the binding (Figure 6I). In addition, Inc-Dpf3 bound to HIF-1α and CT domains primarily in the cytoplasm (Figure 6J), which is in accordance with the subcellular distribution pattern of Inc-Dpf3 (Figures 3A and 3B). On the contrary, the binding of Inc-Dpf3 with the NT1 domain of HIF-1α occurred primarily in the nucleus (Figure 6J), which possibly indicates the function of the small part of Inc-Dpf3 locating in the nucleus.

It has been well known that HIF-1α binds to core nucleotide sequences known as hypoxia response elements (HREs) in the promoter regions of target genes. We wondered whether HIF-1α could interact with HRE elements on IncRNA. Inc-Dpf3 contains one HRE motif within the F4 fragment at 3,866–3,869 nt. We next generated the ΔHRE mutation of Inc-Dpf3 (with AGCGT mutated into AAAAG) and found that HRE-motif mutation partially impaired the ability of F4 fragment to inhibit HIF-1α activity (Figure 7A) and abolished the Inc-Dpf3 binding to HIF-1α (Figure 7B). Adenovirus-mediated overexpression of Inc-Dpf3, but not its ΔHRE mutation, could inhibit CCR7-mediated DC migration and lactate production and rescue the exaggerated DC migration and lactate production in mDCs from Inc-Dpf3ΔHRE-D810A mice (Figures 7C and 7D).

Next, we generated lentiviral vectors overexpressing WT HIF-1α (HIF-1α-WT) or its alanine mutations at two conserved amino acids within the CT domain of HIF-1α: the constitutively inactivate mutation of Asp 810 to Ala (D810A) and the constitutively activate mutation of Asn 814 to Ala (N814A), respectively (Dames et al., 2002) (Figure 7E). Compared to HIF-1α-WT, overexpression of HIF-1α-N814A enhanced DC migration, whereas overexpression of HIF-1α-D810A inhibited DC migration. In addition, the increased migration in Inc-Dpf3ΔHRE-D810A Itgax-cre+ DCs was abolished after overexpression with HIF-1α-D810A (Figure 7F). Therefore, HIF-1α activation was critical for the enhanced DC migration caused by Inc-Dpf3 deficiency. Collectively, these data indicate that Inc-Dpf3 interacts with the N and C termini of HIF-1α via an HRE motif in its 3,000–4,076 nt region and that this motif is important for the activity of Inc-Dpf3 to inhibit HIF-1α and regulate CCR7-mediated DC migration.

**Inc-Dpf3 Suppresses CCR7-Mediated DC Migration via Preventing Glycolytic NAD⁺ Production**

We next wondered whether that increased glycolytic shift in Inc-Dpf3-deficient DCs was needed to provide extra ATP for DC motility. We found that the overall intracellular ATP amounts were increased by CCR7 stimulation (Figure S7E). However, the ATP amounts were slightly lower in Inc-Dpf3-silencing DCs after CCR7 stimulation (Figure S7E), and silencing of Ldha reversed the decreased ATP amounts in Inc-Dpf3-silencing DCs (Figure S7F), indicating that Inc-Dpf3-silencing DCs had reduced amounts of overall ATP due to enhanced Ldhα expression after CCR7 stimulation. Moreover, although inhibition of mitochondrial ATP synthase by oligomycin significantly reduced CCR7-triggered DC migration, Inc-Dpf3-silencing DCs still had increased migratory capacity compared to control DCs, even in the absence of mitochondrial ATP (Figure S6F). Thus, ATP is not the factor that contributes to the enhanced CCR7-triggered migratory activity in Inc-Dpf3-silencing DCs.

Ldhα could catalyze the conversion of pyruvate to lactic acid associated with the oxidation of NADH to NAD⁺ in the final step of aerobic glycolysis. We found that inhibition of Ldhα with oxamate abrogated the effect of Inc-Dpf3 silencing or deficiency in increasing DC migration (Figures S7G and S7H), indicating that Inc-Dpf3 regulates DC migration by inhibiting Ldhα expression. Supplementation of NAD⁺, but not lactic acid (LA), could restore the migration of mDCs in the presence of oxamate (Figures S7G and S7H). These data indicated that the extensive NAD⁺ production mediates the enhanced CCR7-triggered migratory capacity in Inc-Dpf3-silencing or -deficient DCs.

Previous studies suggested NAD⁺ was associated with the dynamic morpho-functional changes of macrophages in response to LPS stimulation (Venter et al., 2014). We therefore suspected that the overproduction of NAD⁺ in CCR7-stimulated DCs may be involved in regulation of the key conductor of leucocyte migration, actin cytoskeleton. Confocal analysis showed that inhibition of glycolysis or Ldhα prevented F-actin polarization induced by CCR7.
stimulation, whereas supplementation of NAD⁺ reversed the effect of oxamate (Figure S7I). Cellular extracts from inc-Dpf3-silencing DCs promoted actin polymerization more efficiently than control DCs (Figure S7J). Accordingly, inhibition of LDHA by oxamate caused a significant reduction in F-actin polymerization, whereas supplementation of NAD⁺ reversed the effect of oxamate (Figure S7K). Therefore, glycolytic NAD⁺ supports DC migration by maintaining F-actin polarization and polymerization, and inc-Dpf3 restrains DC migration by preventing glycolytic LDHA-dependent NAD⁺ production.

Figure 7. inc-Dpf3 Inhibits HIF-1α Activation and CCR7-Triggered DC Migration via an HRE Motif in Its 3,000–4,076 nt Region

(A) HRE luciferase activity in HEK293T cells transfected with indicated plasmids. (B) RIP assay of Flag enrichment at Inc-Dpf3 in HEK293T cells transfected with indicated plasmids. (C and D) mDCs from inc-Dpf3fl/flItgax-cre⁺ and inc-Dpf3fl/flItgax-cre⁻ mice were transfected with adenovirus overexpressing inc-Dpf3 (Ad-inc-Dpf3), HRE mutation of inc-Dpf3 (Ad-inc-Dpf3 ΔHRE), or control adenovirus (Ad-NC). Cells were analyzed for inc-Dpf3 expression to confirm overexpression efficiency (C). CCR7-triggered migration and lactate production were analyzed (D). (E) HRE luciferase activity in lysates of HEK293T cells transfected with lentivirus overexpressing HIF-1α-WT, HIF-1α-D810A, or HIF-1α-N814A. (F) mDCs from inc-Dpf3fl/flItgax-cre⁺ and inc-Dpf3fl/flItgax-cre⁻ mice were transfected with HIF-1α-WT, HIF-1α-D810A, or HIF-1α-N814A lentivirus and then analyzed for CCR7-triggered migration.

Data are from one representative of three independent experiments and are shown as mean ± SD of indicated determinants (A–F). *p < 0.05, **p < 0.01, NS, not significant (unpaired two-tailed Student’s t test).
DISCUSSION

In this study, we identified a CCR7-inducible IncRNA, lnc-Dpf3, whose feedback restrained CCR7-mediated DC migration by inhibiting glycolysis through direct binding and suppressing HIF-1α activity. After CCR7 stimulation, lnc-Dpf3 showed evident upregulation in DCs at the late stage (after 12 h in vitro or after 48 h in vivo). In line with this, lnc-Dpf3 suppressed DC migration at the late stage and restrained inflammatory injuries during CHS responses at 1–3 days. The delayed and inducible function of lnc-Dpf3 in suppressing the late stage of DC migration was essential for the prevention of inflammatory pathogenesis and maintenance of immune balance. It has been shown that resolution of the primary infection results in the development of tolerogenic DCs and macrophages, leading to immune suppression and susceptibility to secondary infections (Roquilly et al., 2017), indicating that DC-based regulatory networks contribute to downregulation of immune responses after clearance of infection. Thus, the magnitude and duration of inflammatory responses are tightly controlled via multiple mechanisms to achieve the balance between immune activation and tolerance.

Emerging evidence indicates a variable role of metabolic reprogramming in immune cell activation and regulation (O’Neill and Hardie, 2013; Du et al., 2018), but the underlying mechanisms are poorly understood. Recently, another group reported that early glycolytic activation in DCs is a common program for stimuli and that inhibition of glycolysis impaired DC migration to draining lymph nodes (Guak et al., 2018). In the current study, through metabolomics profiling, biochemical studies, and functional studies, we demonstrated that CCR7 stimulation induced glycolytic response in DCs via HIF-1α activation, which is restrained by CCR7-inducible lnc-Dpf3 for protection against inflammatory injuries. Therefore, we provide additional models for how signal-induced glycolytic response in turn affects immune cell migration and immune responses and reveal the crosstalk between epigenetic mechanisms and metabolic pathways in feedback-inhibiting inflammatory responses.

Previous studies using high-throughput sequencing as well as cross-linking approaches have revealed a transcriptome-wide map of the distribution of m6A in mRNAs and noncoding RNA (Linder et al., 2015). m6A mRNA methylation has been shown to control T cell homeostasis by targeting the IL-7, STAT5 (signal transducer and activator of transcription 5), and SOCS (suppressor of cytokine signaling) pathways (Li et al., 2017). Our previous work has shown that DDX46-mediated erasure of the m6A methylation of antiviral mRNA transcripts is important for inhibition of interferon production and antiviral immunity (Zheng et al., 2017). However, the formation, recognition, and regulation of this modification in specific IncRNA, especially their role in immune responses, have been poorly understood. It was recently shown that the long non-coding RNA X-inactive specific transcript (XIST) mediates its gene repression function in a manner dependent on m6A formation and recognition (Patil et al., 2016). We show here that CCR7-induced downregulation of m6A modification increased Inc-Dpf3 expression and therefore enabled Inc-Dpf3-mediated suppression of DC migration and prevented exaggerated inflammatory responses. Therefore, we have identified a dynamic pattern of m6A modification in post-transcriptionally regulating IncRNA stability during innate immune responses.

STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found with this article online at https://doi.org/10.1016/j.immuni.2019.01.021.

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AUTHOR CONTRIBUTIONS

X.C. designed and supervised the research; J.L., X.Z., K.C., Y.C., S.L., M.X., Y.C., H.Z., and Z.L. performed experiments; J.L., X.Z., K.C., and X.C. analyzed data; J.L. and X.C. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES


### STAR METHODS

#### KEY RESOURCES TABLE

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Chemicals, Peptides, and Recombinant Proteins

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Critical Commercial Assays

| Glucose Uptake Colorimetric Assay Kit | Biovision | Cat# K676-100 |
| Lactate Colorimetric Assay Kit II    | Biovision | Cat# K627-100 |
| NAD⁺*/NADH Quantification Colorimetric Kit | Biovision | Cat# K337-100 |
| ATP Determination Kit                | Beyotime Biotech | Cat# S0027 |
| JC-1 assay kit                      | Beyotime Biotech | Cat# C2006 |
| Actin Polymerization Biochem kit     | Cytoskeleton | Cat # BK003 |
| SMARTer RACE 5'/3' Kit               | Clontech | Cat# 634558 |
| Chemiluminescent EMSA Kit           | Beyotime Biotech | Cat# G509 |
| ChIP assay kit                      | Millipore | Cat# 17-371FR |
| Magna RIP RNA-Binding Protein Immunoprecipitation Kit | Millipore | Cat# 17-700 |
| Duolink In Situ Red Kit Mouse/Rabbit | Sigma Aldrich | Cat# DU92101 |
| T7 RNA Polymerase transcription kit  | Promega | Cat# P2075 |
| PrimeFlow RNA Assay Kit              | Thermo Fisher | Cat# 88-18005-210 |
| LightShift Chemiluminescent EMSA Kit | Thermo Fisher | Cat# 20148 |

Deposited Data

| IncRNA-seq raw data                | This paper | GSE118533 |
| mRNA-seq raw data                  | This paper | GSE118531 |

Experimental Models: Cell Lines

| HEK293T                           | ATCC     | Cat# CRL-11268; RRID: CVCL_1926 |

Experimental Models: Organisms/Strains

| C57BL/6 mice                       | Shanghai Laboratory Animal Center, Chinese Academy of Science | N/A |
| Itgax-cre mice                     | The Jackson Laboratory | JAX 008068; RRID: IMSR_JAX:008068 |

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### Oligonucleotides

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Recombinant DNA

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lnc-Dpf3 RNA flow probe #2 (LE): aataggtctctgtgatgag tacc (5'-3')

lnc-Dpf3 RNA flow probe #3 (LE): ttaactgtaactcagctcaaa cgttaaag (5'-3')

lnc-Dpf3 RNA flow probe #4 (LE): ggtcacagatggctatag agatgc (5'-3')

lnc-Dpf3 RNA flow probe #5 (LE): gccagagaagcttgtcttt gtgat (5'-3')

lnc-Dpf3 RNA flow probe #6 (LE): gcgctgctgtccctgttg (5'-3')

lnc-Dpf3 RNA flow probe #7 (LE): ccgttgatgcccatgg tga (5'-3')

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### Software and Algorithms

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CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Xuetao Cao (caoxt@immunol.org).

METHOD DETAILS

Mice
C57BL/6 mice (6–8 weeks of age) were obtained from the Shanghai Laboratory Animal Center, Chinese Academy of Science. Inc-Dpf3 floxed mice were generated using ES cell targeting and homologous recombination. A Inc-Dpf3 targeting plasmid vector was constructed by ET cloning techniques in EL250 bacterial cells (Liu et al., 2003), based on the BAC clone RPCI-23-451D21 (Source BioScience Life Sciences). The targeting vector was designed to flank the mouse Inc-Dpf3 DNA region with loxp sites and a pGK-Neomycine-polyA cassette. The targeting vector was confirmed by sequencing and electroporated into C57BL/6J embryonic stem cells (ES), which were then subjected to double drug selection with G418 and ganciclovir for screening the homologous recombination clones. The resistant ES clones were identified by long PCR and confirmed by sequencing. The primers used for genotyping the correct homology recombination were 5′-CTTATGCTAGGCGGACACTC-3′ and 5′-GATCACCCTCTAACTAACC-3′ for the correct 5′ homology arm recombination, and 5′-TCGCATTGTCTGAGTAGGTGTC-3′ and 5′-CATCAGCACCAGCATAAATCAGA-3′ for the correct 3′ homology arm recombination. Positive ES cell clones were expanded and injected into C57BL/6J blastocysts to generate the chimeric offspring. The chimeric mice were mated with C57BL/6J mice to obtain the Inc-Dpf3 floxed heterozygous mouse (Inc-Dpf3 fl/+). The Inc-Dpf3 fl/+ mice were bred with C57BL/6J mice expressing the Cre recombinase under the control of the Itgax promoter (Itgax-cre mice; The Jackson Laboratory) to generate Inc-Dpf3 floxed heterozygous mouse with Itgax-cre allele (Inc-Dpf3 fl/+Itgax-cre mice). These mice were intercrossed to generate DC-specific Inc-Dpf3-deficient mice (Inc-Dpf3 fl/flItgax-cre) used for this study. Hif1a fl/fl mice, a kindly gift from Dr. Ming Zhang (Renji Hospital, Medical College of Shanghai Jiaotong University, China) were crossed with Itgax-cre mice to generate DC-specific HIF-1α-deficient mice (Hif1a fl/flItgax-cre). All mice were maintained under specific pathogen-free conditions and used at 6–8 weeks of age. All animal experiments were carried out according to National Institute of Health Guide for the Care and Use of Laboratory Animals, with the approval of the Scientific Investigation Board of Second Military Medical University (China).

Cell Isolation and Culture
Bone marrow cells were cultured with recombinant mouse GM-CSF (10 ng/ml) and IL-4 (1 ng/ml) for generation of BMDC as previously described (Liu et al., 2014). On day 6 of culture, the CD11c+ BMDC isolated by positive selection with anti-mouse CD11c magnetic beads were collected as immature DC (imDC). imDC were further stimulated with LPS (100 ng/ml) for 24 h to generate mature BMDC (mDC). CD4+ T cells, CD19+ B cells, DX5+ NK cells and splenic CD11c+ DC were respectively enriched from splenocytes via positive selection with corresponding magnetic beads. HEK293T cells was from American Type Culture Collection and used for plasmid transfection.

Transwell Migration Assay
The migration assay was performed using 24-well Transwell plates containing 8-μm-pore size polycarbonate filters (Corning, Life Science). CCL19 and CCL21 (50 ng/ml in a total volume of 600 μl) was added to the lower chambers. mDC were added to the upper chamber (1 × 10⁶ cells in a total volume of 100 μl) and incubated for 12 h at 37°C. In some experiments, DC were pretreated with vehicle or AKT-IV (10 μM), Wortmannin (50 μM), PD98059 (100 μM), Rapamycin (100 μM), 2-ME (10 μM), 2-DG (100 mM), oligomycin (1 μM), oxamate (50 mM), NAD+ (100 μM), or lactic acid (25 mM) for 1 h. The number of migrated DC into the lower chamber was determined by flow cytometry. The number of spontaneously migrated DC in the absence of chemokine was subtracted as background.

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**In Vivo Migration of DC**
mDC were labelled with 0.5 mM of carboxyfluorescein diacetate succinimidy ester (CFSE). A total of $2 \times 10^6$ labeled cells was injected subcutaneously in the hind leg footpad of WT recipient mice. Popliteal lymph nodes were collected at indicated time after DC administration, the proportion of CFSE$^+$ CD11c$^+$ DC was evaluated by flow cytometry.

**Metabolism Assays**
For real-time analysis of the ECAR and OCR, mDC were pretreated with vehicle or AKT-IV (10 μM), 2-ME (10 μM) for 1 h and stimulated with CCL19 and CCL21 (50 ng/ml) for indicated times. For real-time analysis of the ECAR and OCR, mDC were analyzed with an XF-96 Extracellular Flux Analyzer ( Seahorse Bioscience) as described. Glycolytic stress test was performed using the following injection strategy, 25 mM glucose, 1 mM oligomycin, and 100 mM 2-DG. The cellular glucose uptake, lactate production and ratio of NAD$^+$ to NADH were determined with Glucose Uptake Colormetric Assay Kit (Biovision), Lactate Colormetric Assay Kit II (Biovision), NAD$^+$ to NADH Quantification Colorimetric Kit (Biovision) respectively, according to each manufacturer’s recommendations. Intracellular ATP concentrations were determined with an ATP Determination Kit according to the manufacturer’s instructions (Beyotime Biotech, China). Cellular mitochondria were stained with Mito-Tracker Green (Beyotime Biotech, China) and determined with Flow cytometry. Mitochondria membrane potential was determined using JC-1 assay kit (Beyotime Biotech, China). For unbiased metabolomics analysis, mDC (5 × 10$^5$ cells) were stimulated with CCL19 and CCL21 (50 ng/ml) for 12 h and subjected to metabolite profiling using gas chromatography time-of-flight mass spectrometry (GC-TOF/MS) system by Metabo-Profile, Shanghai, China. Data are analyzed using a XploreMET v3.0 system (Metabo-Profile, Shanghai, China).

**Flow Cytometry**
For cell surface staining, the single-cell suspensions were incubated with the antibody cocktails for 20 min at 4°C. For intracellular cytokine staining, cells were fixed with 4% paraformaldehyde for 20 min at 4°C and subsequently permeabilized in Perm buffer in the presence of antibodies overnight at 4°C. Data were obtained on an LSR II and analyzed with FACSDiva software (BD Biosciences).

**Confocal Microscopy**
mDC were pretreated with vehicle or 2-DG (100 mM), oxamate (50 mM) or NAD$^+$ (100 μM) for 1 h and then stimulated with CCL19 and CCL21 (50 ng/ml) for 12 h. Cells were fixed with 4% paraformaldehyde for 30 min at 4°C and then permeabilized in PBS containing 0.5% Triton X-100 for 10 min at room temperature. Cells were then stained with FITC-Phalloidin in the presence of 1% BSA for 30 min and finally incubated with DAPI for 10 min at room temperature. Cells were washed 3 times with 1×PBS for 5 min between each incubation step. Images were obtained with laser scanning confocal microscope (Leica TCS SP8) and analyzed by the LAS X software version 2.0.2.15022.

**Time-Lapse Imaging of DCs**
Time-lapse imaging of mDC were conducted using EZ-TAXIScan chamber (Effector Cell Institute, Tokyo, Japan) assembled with a 260 μm wide × 4μm thick silicon chip according to manufacturers’ instructions. In brief, mDC were added to the lower reservoir and 1 μl of chemoattractant (CCL19 or CCL21 at a concentration of 50 ng/ml) to the upper reservoir. The migration of mDC at room temperature was recorded every 30 s for 4 h and the velocity of migrated DCs was analyzed with TAXIScan Analyzer 2 software.

**F-actin Polymerization Assay**
F-actin polymerization assay were performed using an Actin Polymerization Biochem kit (Cytoskeleton) according to the manufacturer’s instructions. In brief, mDC pretreated with vehicle or oxamate (50 mM) or NAD$^+$ (100 μM) for 1 h and then stimulated with CCL19 and CCL21 (50 ng/ml) for 12 h. Cell extracts were collected as test samples. The effects of test samples on actin polymerization were determined by the fluorescence enhancement compared to the baseline. The relative fluorescence unit (RFU) in each well was measured at Excitation wavelength 350 nm, Emission wavelength 407 nm by EnSpire Workstation (version 4.13.3005.1482).

**Quantitative Real-Time RT-PCR**
Total RNA was extracted from cultured cells with TRIzol reagent according to the manufacturer’s instructions. RNA was reverse transcribed with Oligo(dT) primer for mRNA and random primer for IncRNA into cDNA with M-MLV Reverse Transcriptase, RNase H- (Takara), RNA expression was quantified by realtime PCR with SYBR Premix ExTaq kit (Takara) and normalized to the expression of β-actin for mRNA or U6 for lncRNA. Results are then relative to those in the control group, set as 1. Amplification of cDNA was performed on the Light Cycler instrument (Roche Diagnostics). qRT-PCR primers used for detecting expression of indicated IncRNAs and mRNAs are listed in KEY RESOURCES TABLE.

**Immunoblot Analysis**
imDC or mDC were stimulated with CCL19 and CCL21 (50ng/ml) for indicated time and lysed using M-PER Protein Extraction Reagent ( Pierce), supplemented with protease inhibitor cocktail (Calbiochem). Protein concentrations were determined with bicinchoninic acid assay (Pierce). Equalized extracts were used for immunoblot analysis as previously reported (Chen et al., 2017).
nucleus and cytoplasmic isolation, cells were lysed in 0.1% NP40 ice-cold PBS with protease inhibitor cocktail (Calbiochem) and Ribonucleoside Vanadyl Complex (10mM) (New England BioLabs). After short centrifugation, the supernatant was collected as cytoplasmic fraction and the remainder with additional washing was considered as nuclear pellets.

**RNA Interference**

Interference of IncRNAs were conducted with the Ribo™ Smart Silencer designed and synthesized in Ribobio (China). Each Ribo™ Smart Silencer contains three siRNA and three antisense oligonucleotides targeting different sequences. siRNA for mouse Hif1a, Ythdf1, Ythdf2, Ldha, and Dpf3 were designed and synthesized by the GenePharma (China). mDC were transfected with siRNA using INTERFERin reagent (Polyplus) following manufacturer’s instructions. siRNA targeted sequence of lncRNAs and siRNA sequence specific for indicated genes are listed in KEY RESOURCES TABLE.

**Overexpression Vectors and Transfection**

The cDNA for Inc-Dpf3 and ΔHRE mutation of Inc-Dpf3 were amplified by PCR and subcloned into the adenovirus shuttle plasmids (AdMax™; Microbix Biosystems, Toronto, Canada). The shuttle plasmids were then cotransfected into HEK293T cells with adenoviral genomic plasmids. The cDNA for Dpf3 (NM_001267625), HIF-1α (NM_001313919.1), as well as their respective fragments and mutations were constructed by PCR-based amplification from mDC cDNA, followed by subcloning into the pcDNA3.1/Flag eukaryotic expression vector (Invitrogen). The HEK293T cell line (American Type Culture Collection) was transfected with JetPEI and Flag-tagged mouse Hif1a (NM_001313919.1), as well as their respective fragments and mutations were constructed by PCR-based amplification from mDC cDNA, followed by subcloning into the pcDNA3.1/Flag eukaryotic expression vector (Invitrogen). The HEK293T cell line (American Type Culture Collection) was transfected with JetPEI reagents (Polyplus) following the instructions. RIP and RNA pull-down analysis was conducted 36-48 h after transfection.

**Plasmid Constructs and Transfection**

The recombinant vectors encoding Inc-Dpf3 and ΔHRE mutation of Inc-Dpf3 were amplified by PCR and subcloned into the pcDNA3.1/Flag expression vector (Invitrogen). The HEK293T cell line (American Type Culture Collection) was transfected with adenovirus shuttle plasmids and mutations were constructed by PCR-based amplification from mDC cDNA, followed by subcloning into the pcDNA3.1/Flag eukaryotic expression vector (Invitrogen). The HEK293T cell line (American Type Culture Collection) was transfected with JetPEI reagents (Polyplus) following the instructions. RIP and RNA pull-down analysis was conducted 36-48 h after transfection.

**Dual-Luciferase Reporter Assay**

HEK293T cells (5×10⁴ cells/well, 96 well plate) were transiently transfected with plasmids encoding Inc-Dpf3 or its fragments and mutations as indicated, and Hif1a as well as HRE driven luciferase reporter and Renilla luciferase reporter vector (Promega) with Vetogene HD (Promega). In some experiments, cells were stimulated with CCL19 and CCL21 (50ng/ml) for indicated time after transfection. Luciferase expression was determined by measuring luminescence with the Dual-Luciferase Reporter Assay System (Promega). The firefly luciferase activity was normalized to Renilla luciferase activity.

**Electrophoretic Mobility Shift Assay (EMSA)**

Nuclear extracts from mDC stimulated with CCL19 and CCL21 (50ng/ml) for 12 h were incubated with a biotin-labeled probe specific for HIF-1α DNA-binding sites (5′-TCCTGACAGCACCCTACACTCC-3′; 3′-AGACATGCACTGATGAGTTGAGG-5′) (Betoytime Biotech, China). The labelled samples were subjected to electrophoresis using Chemiluminescent EMSA Kit (Betoytime Biotech, China) according to the instructions.

**Chromatin Immunoprecipitation (ChIP)**

ChIP analyses were performed with anti-HIF-1α antibody and the assay was performed using ChIP assay kit (Millipore) according to manufacturer’s instructions. HIF-1α enrichment was quantified using quantitative RT-PCR and expressed as fold change to input. Primers for amplification of Ldha gene promoters are listed in the KEY RESOURCES TABLE.

**Rapid Amplification of Cloned cDNA Ends (RACE)**

Total RNA extracted from mouse mDC was subjected to RACR PCR with SMARTer RACE 5′-3′ Kit according to the manufacturer’s protocol (Clontech). Primers used for Inc-Dpf3 5′ RACE and 3′ RACE are listed in the KEY RESOURCES TABLE. Inc-Dpf3 sequence obtained from RACE (5′-3′, 4076 nt)
RNA Immunoprecipitation (RIP) qRT-PCR

RNA immunoprecipitation (RIP) was performed with antibodies specific to HIF-1α, p-p65, Ythdf1, Ythdf2, Flag or IgG by using Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore) according to the manufacture’s protocol. m6A qRT-PCR was performed as described previously (Zheng et al., 2017). Fold enrichment was quantified using quantitative RT-PCR and expressed as fold change to input. Primers used for detecting m6A enrichment in specific lnc-Dpf0 region in m6A RNA immunoprecipitation (Me-RIP) are listed in the KEY RESOURCES TABLE.

RNA Pull-Down Assay

Biotin-labeled sense or antisense lnc-Dpf3 and lnc-Dpf3 fragments were transcribed in vitro using T7 RNA Polymerase transcription kit (Promega) and Biotin RNA Labeling Mix (Roche). The isolated biotin-labeled RNA was heated to 90°C for 2min and placed on ice for 2min. The folded RNA were then added to the lysate HEK293T cells transfected with Flag-tagged HIF-1α and incubated at room temperature for 1h. 100 μl washed streptavidin-coupled beads were added to the reaction and rotated at 4°C for 1h followed. The beads were separated by SDS-PAGE followed by immunoblot identification.

RNA Fluorescence In Situ Hybridization (FISH)

Biotin-conjugated lnc-Dpf3 probes for FISH assay were designed by the Stellaris FISH Probe Designer (Biosearch Technologies). mDC were fixed in 4% formaldehyde plus 10% acetic acid in PBS for 15 min at room temperature, and then were permeabilized in PBS plus 0.5% Triton X-100 for 10 min on ice. Hybridization was carried out using lnc-Dpf3 probe sets according to the protocol of Biosearch Technologies. Cells were further incubated with rabbit anti-HIF-1α antibody followed by Alexa Fluor 488 anti-rabbit secondary antibody. Images were obtained and analyzed as in the confocal microscopy.
RNA In Situ Hybridization Proximity Ligation Assay (rISH-PLA)

The protocol of rISH-PLA to detect the interaction of Inc-Dpf3 and HIF-1α were modified from the previously reported ISH-PLA method (Gomez et al., 2013), using the Duolink In Situ Red Kit Mouse/Rabbit (Sigma Aldrich). In brief, HEK293T cells transfected plasmid overexpressing Inc-Dpf3 and Flag-tagged HIF-1α were fixed with 4% paraformaldehyde for 10 min and subsequently permeabilized with 0.5% Triton X-100 for 5 min at room temperature. After hybridization with biotin-conjugated Inc-Dpf3 probes at 37°C overnight, cells were incubated with mouse anti-biotin antibody and rabbit anti-Flag antibody at 37°C for 30 min. Then two secondary PLA (MINUS and PLUS) probes conjugated to oligonucleotides are then added to cells and incubated at 37°C for 1 h. The ligation solution consisting of two oligonucleotides and ligase were then added and incubated at 37°C for 30 min. The amplification solution consisting of nucleotides and fluorescently labeled oligonucleotides and polymerase were next added and incubated at 37°C for 100 min. Red fluorescent dots (indicating close proximity of RNA and protein) were imaged and analyzed as in the confocal microscopy.

RNA Flow Cytometry

Inc-Dpf3 expression within distinct DC subsets were detected using PrimeFlow RNA Assay Kit (Thermo Fisher) according to the manufacturer’s instructions. In brief, singe cell suspensions of dLNIs were subjected to surface marker staining, and then incubated with Inc-Dpf3-specific label extender (LE) and blocker (BL) probes. The cells were then hybridized with Pre-Amplifier and Amplifier DNA (Alexa Fluor 647) and incubated with label probes. Cells were finally detected using an LSR Fortessa flow cytometer (BD biosciences).

FITC Skin Painting

Inc-Dpf3<sup>fl/fl</sup>/Itgax-cre<sup>+</sup> mice, Hif1α<sup>fl/fl</sup>/Itgax-cre<sup>+</sup> mice, and their respective control mice were painted on the shaved abdomen with 0.1 ml FITC (Invitrogen) at 5 mg/ml dissolved in a 50:50 (vol/vol) acetone–dibutylphthalate mixture (Sigma Aldrich) or the vehicle alone as control. After 48 h, inguinal lymph nodes were collected and analyzed for FITC<sup>+</sup>CD11c<sup>+</sup> DC by flow cytometry.

Induction of Contact Hypersensitivity (CHS) Responses

CHS was induced as described previously with minor modifications (Natsuaki et al., 2014). Inc-Dpf3<sup>fl/fl</sup>/Itgax-cre<sup>+</sup> and control mice were sensitized on shaved abdominal skin with 50 μl 0.5% (wt/vol) DNFB (1-fluoro-2,4-dinitrofluorobenzene; Sigma Aldrich) dissolved in acetone and olive oil (at a ratio of 4:1). After 5 days, the right ear was challenged with 10 μl 0.2% DNFB while the left ear received the vehicle alone. CHS response was determined by measuring the net change of ears at 12-72 h after challenge using a digital display Vernier caliper (91511; Sata, China). Ear histology was examined at day 3 after induction.

In some experiments, CHS response was evaluated after in vivo inoculation of antigen-loaded DC. In brief, mDC were treated with DNBS (100 μg/ml) at 37°C for 30 min. For sensitization, 1×10<sup>6</sup> DNBS pulsed mDC diluted in 200 μl saline were injected subcutaneously into the flank of WT recipient mice. After 5 days, mice were challenged by the application of DNFB (0.2%) on the right ear or vehicle on the left ear as control. Ear thickness was analyzed at 12-72 h after DNFB challenge, and ear histology were examined at day 3.

RNA Decay Assays

To detect the lifetime of Inc-Dpf3, cells were incubated with actinomycin D (5 mg/ml) to terminate transcription. RNA was collected at 0 and 6 h post transcription termination. RNA samples were collected and the remaining Inc-Dpf3 was determined by RT-PCR.

mRNA Sequencing and IncRNA Sequencing

Total RNA was isolated from Inc-Dpf3-silencing and control mDC stimulated with CCL19 and CCL12 (50 ng/ml) for 12 h and subjected to cDNA library construction and RNA sequencing by Beijing Genomics Institute. In brief, mRNA sequencing was conducted using BGISEQ-500 platform and the high-quality reads were aligned to the mouse reference genome (GRCm38). IncRNA sequencing were conducted using Illumina HiSeq platform and the high-quality reads were aligned to publicly available NONCODE database. Gene expression were normalized to fragments per kilobase of exon model per million mapped reads (FPKM) by Expectation Maximization (RSEM).

Statistical Analysis

Statistical significance was assessed by unpaired two-tailed Student’s t test with a value of p < 0.05 considered to be statistically significant (*p < 0.05, **p < 0.01). The statistical tests were justified as appropriate according to assessment of normality and variance of the distribution of the data. No randomization or exclusion of data points was used. No ‘blinding’ of investigators was applied. Sample size was chosen according to previous experience and preliminary studies to ensure adequate power.

DATA AND SOFTWARE AVAILABILITY

The source data that support the findings of this study are available from the corresponding author upon request. The accession numbers for the mRNA sequencing data and IncRNA sequencing data reported in this paper are GenBank: GSE118531 and GSE118533, respectively.