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RESEARCH ARTICLE

Kinesin family member KIF18A is a critical cellular factor that regulates the differentiation and activation of dendritic cells

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Abstract

Background KIF18A is a kinesin family member that is involved in various cellular processes including cell division, cell transformation, and carcinogenesis. However, its possible role in the regulation of host immunity has not been examined.

Objective The aim of this study is to investigate the functional role of KIF18A in the differentiation and activation of dendritic cells (DCs) that are the most efficient antigen-presenting cells.

Methods A bioinformatic analysis of the KIF18A gene family was performed to understand its sequence variability and evolutionary history. To inhibit KIF18A activity, a highly specific small molecule inhibitor for KIF18A, BTB-1 was used. DCs were differentiated from mouse bone marrow (BM) cells from 6 to 7 week old C57BL/6 mice with recombinant granulocyte–macrophage colony-stimulating factor (GM-CSF). Expression of KIF18A was measured by Western blotting. The surface expression of differentiation and activation markers on DCs were analyzed by flow cytometry.

Results The phylogenetic analysis revealed that the KIF18A gene family is remarkably conserved across vertebrates. Interestingly, the expression of KIF18A was increased as BM precursor cells differentiated into DCs. BTB-1 treatment strongly inhibited the differentiation of BM cells into DCs in a dose-dependent manner. Furthermore, treatment of immature DCs with BTB-1 significantly impaired the expression of activation markers on DCs including MHC class I, CD80, and CD86 upon TLR4 or TLR7 treatment.

Conclusion Our results reveal that KIF18A is a critical DC differentiation and activation regulator. Therefore, KIF18A could be a potential therapeutic target for immune-mediated disorders.

Keywords KIF18A · Phylogenetic analysis · Dendritic cells · Toll-like receptor

Introduction

Kinesins are motor proteins that hydrolyze ATP to move along microtubules (Yildiz and Selvin 2005). They are known to play important roles in cell division, chromosome alignment, cargo transport, and maintenance of cell morphology (Du et al. 2010; Jordan and Wilson 2004; Weaver et al. 2011). Interestingly, these proteins are associated with infectious diseases because they regulate the activation and differentiation of immune cells. For example, Eg5-kinesin is essential in the NK cell initiation of the microbactericidal granule movement (Ogbomo et al. 2018). Also, kinesin family members KIF4 and KIF7 are known to regulate the activation and development of T cells (Bernasconi et al. 2008; Lau et al. 2017). Therefore, kinesins could be important therapeutic targets for immune-mediated disorders. One kinesin family member, KIF18A, is known to control spindle length and the alignment of mitotic chromosomes at the spindle equator during cell division (Weaver et al. 2011). Interestingly, KIF18A is involved in several forms of cancer, including colorectal, breast, and hepatocellular (Liao et al. 2014; Mayr et al. 2011; Nagahara et al. 2011; Zhang et al. 2010). However, its role in host immunity is not yet defined.

Dendritic cells (DCs) are the most efficient antigen-presenting innate immune cells, and they are differentiated from several types of precursor cells including hematopoietic stem cells in bone marrow and monocytes in peripheral blood

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(Caux et al. 1997; Sallusto and Lanzavecchia 1994). Most DCs reside in peripheral tissues in an immature state where they recognize pathogens via pattern recognition receptors (PRRs) (Kawai and Akira 2006, 2010). Activation of PRRs such as Toll-like receptors (TLR) leads to the maturation of DCs as well as increased expression of stimulatory molecules and enhanced migration capability (Kawai and Akira 2010). Mature DCs in secondary lymphoid organs such as lymph nodes and the spleen activate naïve T cells, which leads to the initiation of adaptive immune responses (Banchereau et al. 2000). Therefore, the manipulation of DCs is a promising immunotherapeutic strategy to treat diverse immune-mediated diseases.

In this study, we investigated the role of KIF18A in the differentiation and activation of DCs. We found that KIF18A expression was increased in DCs when compared to their precursor cells. Treatment of bone marrow (BM) precursor cells with a KIF18A-specific inhibitor significantly impaired their ability to differentiate into DCs. Furthermore, the activation of DCs was dramatically attenuated by inhibition of KIF18A. These results indicate that KIF18A is an essential cellular factor controlling the differentiation and activation of DCs.

Materials and methods

Orthologs and phylogenetic analysis

A similarity search was performed using the Basic Local Alignment Search Tool (BLAST, ver. 2.9.0) program (Camacho et al. 2009) with the human KIF18A protein sequence as a query. Twenty-one KIF18A gene candidates were obtained from the non-redundant (NR) protein database at the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov). A KIF18A gene candidate from a sea lamprey (*Petromyzon marinus*) was downloaded from the SIMRbase Genome (https://genomes.stowers.org). The species and the accession numbers are listed in Fig. 1. Multiple sequence alignments were generated using MAFFT (ver. 7.407) with the L-INS-I algorithm (1000 maxiterate and 100 retree). Phylogenetic analysis was performed using RAxML (ver. 8.2.12) with the PROTGAMMAILG model. Bootstrapping with 1000 pseudo-replicates was performed to estimate the confidence of the branching patterns for the maximum-likelihood and neighbor-joining methods. Phylogenies were presented with FigTree software (http://tree.bio.ed.ac.uk/software/figtree).

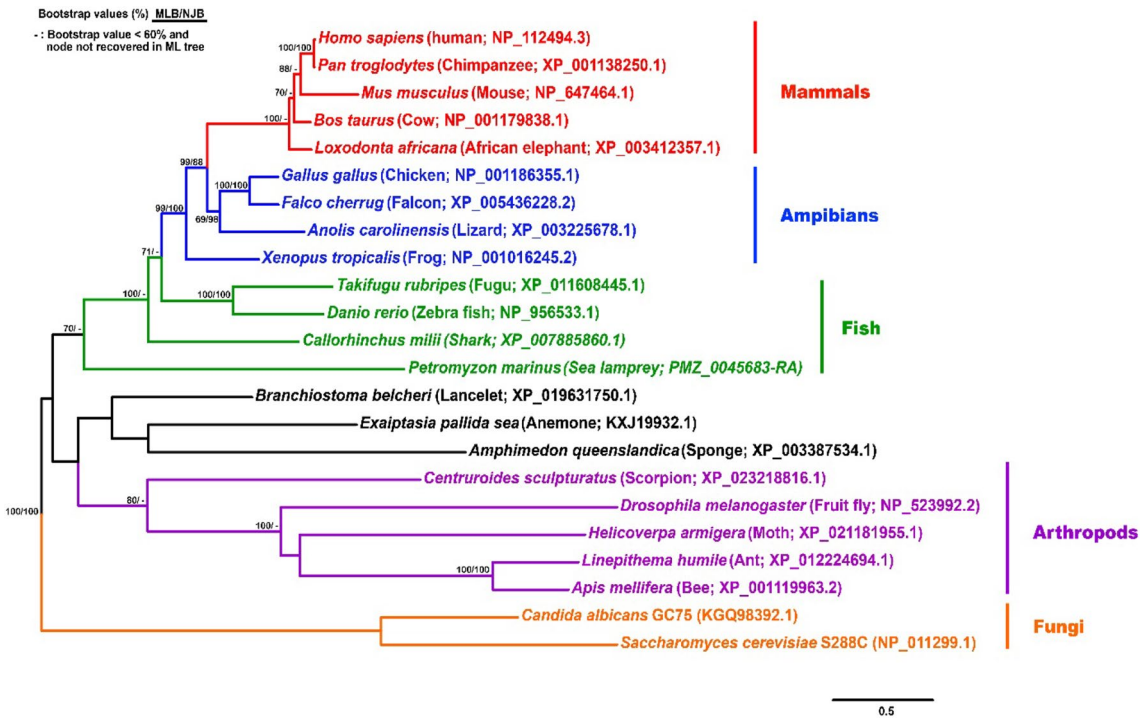

Fig. 1 Phylogenetic analysis of KIF18A in eukaryotes. The maximum-likelihood phylogeny of KIF18A candidate proteins from 21 eukaryotic organisms is shown. Two fungi sequences (*Saccharomyces cerevisiae* S288C and *Candida albicans* GC75) are used as the outgroup. Red-colored, blue-colored, green-colored, and purple-colored branches indicate mammals, amphibians, fish, and arthropods, respectively. The numbers at internal branches show the bootstrap support values (%) for the maximum-likelihood phylogeny (left) and the neighbor joining phylogeny (right). Supporting values are shown only for the internal branches that have at least one method supporting higher than 60%. The scale bar represents the number of amino acid substitutions per site (color figure online)

Reagents and antibodies

The KIF18A inhibitor, BTB-1, was purchased from Tocris Bioscience (MN, USA). Lipopolysaccharides (LPS) and resiquimod (R848) (agonists of TLR4 and TLR7, respectively) were purchased from Sigma-Aldrich (MO, USA). Antibodies specific for KIF18A and actin were purchased from Abcam (UK) and Cell Signaling Technology (MA, USA), respectively. Anti-mouse CD11b, CD11c, CD86, MHC class II, and MHC class I antibodies were purchased from Tonbo Biosciences (CA, USA).

Dendritic cell generation

To generate bone marrow-derived dendritic cells (BMDCs), bone marrow cells were harvested from 6 to 7 week old C57BL/6 mouse femurs and tibias. After lysis of red blood cells with ACK lysis buffer (Thermo Fisher Scientific, MA, USA), cells were cultured in RPMI-1640 (Welgene, Korea) containing 10% fetal bovine serum (FBS, Hyclone), 1% penicillin/streptomycin (Welgene, Korea), and 20 ng/ml GM-CSF (Peprotech, NJ, USA) for 6 days.

Western blot analysis

Cell lysates were prepared using Protein Extraction Solution (Elpisbio, Korea) supplemented with protease and phosphatase inhibitors (Thermo Fisher Scientific, MA, USA). Protein concentrations were measured using the smart BCA protein assay kit (Intronbio, Korea). 5 μg of protein were loaded onto 10% SDS–PAGE gels. Proteins were transferred onto nitrocellulose membranes (GE Healthcare, PA, USA), followed by blocking for 1 h at room temperature in TBS-Tween 20 solution containing 2% BSA. Membranes were then incubated with anti-KIF18A or anti-actin antibodies. SuperSignal West Pico Plus enhanced chemiluminescent substrate (Thermo Fisher Scientific, MA, USA) was used to detect protein bands. Densitometry of bands was performed with scanned images using Image J (NIH, Bethesda, MD, USA).

Flow cytometric analysis

DCs were harvested and resuspended in flow cytometry buffer (1% BSA, 2 mM EDTA, and 0.1% sodium azide in PBS). Cells were then stained with anti-mouse CD11b-PerCP Cy5.5, CD11c-FITC, CD86-APC, or MHC class II-PE antibodies for 20 min at 4 °C. Data were acquired on the Attune™ NxT Acoustic Focusing Cytometer (Thermo Fisher Scientific) and analyzed with Flowjo software (BD biosciences, CA, USA).

Statistical analysis

Error bars indicate the standard error of the mean (SEM). Statistical analyses were performed using Student's *t* test. All experiments were repeated independently at least three times.

Results

KIF18A genes are present in Eukaryotes

We obtained twenty-three representative KIF18A homologous proteins from eukaryotic animals and performed a phylogeny analysis to understand the origin of the KIF18A gene family and their evolutionary patterns. The phylogenetic tree was supported by maximum likelihood bootstrap replicates. The maximum likelihood tree was nearly congruent with the current eukaryotic phylogeny with the black-colored branches as exceptions (Fig. 1). This result indicates that KIF18A genes are highly conserved across vertebrates including *Petromyzon marinus* (sea lamprey).

KIF18A expression was increased in DCs

While the innate immune system has been found in both vertebrates and invertebrates, the adaptive immune system has been found only in vertebrates (Litman et al. 2010). Interestingly, the phylogenetic analysis revealed that KIF18A genes are evolutionarily conserved (Fig. 1) across vertebrates, suggesting its possible involvement in the adaptive immunity. Since DCs are principal immune cells that initiate adaptive immune responses, we hypothesized that KIF18A plays an important role in DC differentiation and function. To test this hypothesis, we first compared KIF18A expression levels between DCs and their precursor cells. BM cells were cultured in the presence of granulocyte–macrophage colony-stimulating factor (GM-CSF) for 0 and 3 days to analyze KIF18A protein expression with Western blotting. KIF18A protein expression was significantly increased at day 3 when compared to its expression at day 0 (Fig. 2a, b), indicating that KIF18A is involved in the process of DC differentiation.

Inhibition of KIF18A interfered with the differentiation of BM cells into DCs

Since KIF18A expression increased during DC differentiation (Fig. 2), we tested whether blocking KIF18A activity influences DC differentiation. BM cells were either untreated or treated with various doses of the selective

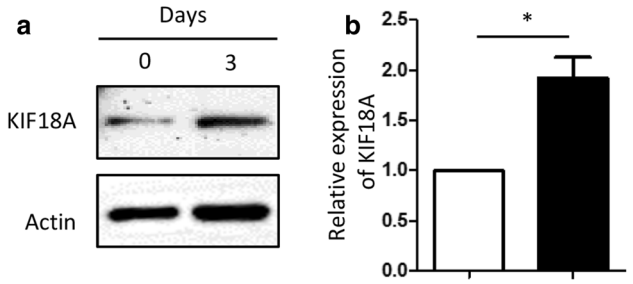

Fig. 2 KIF18A expression is increased during DC differentiation. Mouse bone marrow (BM) cells were cultured in the presence of GM-CSF (20 ng/mL) for 0 and 3 days. Expression of KIF18A and actin was analyzed by Western blot analysis. Changes in the density of bands relative to those of actin are presented. Error bars represent means ± SEM of three independent experiments (*$P < 0.05$)

KIF18A inhibitor, BTB-1, in the presence of GM-CSF. The frequency of CD11c$^+$/MHC II$^+$ cells was significantly reduced in a BTB-1 dose-dependent manner at day 3 (0 μM, 14.5%; 1.25 μM, 10.1%; 2.5 μM, 7.06%; 5 μM, 4.16%; and 10 μM, 2.87%) (Fig. 3a). In addition, the expression of the co-stimulatory molecule, CD86, significantly decreased in a dose-dependent manner when cells were treated with BTB-1 (Fig. 3b). Consistent with these results, the frequency of CD11c$^+$/MHC II$^+$ cells (Fig. 3c) and the expression of CD86 (Fig. 3d) were reduced at the 6-day time point, as well. These results collectively suggest that KIF18A is a critical cellular factor that regulates the differentiation of BM cells into DCs.

Inhibition of KIF18A activity attenuated activation of DCs

We next examined whether KIF18A affects the activation states of DCs. To this end, BMDCs were either untreated or treated with BTB-1 in conjunction with TLR4 or TLR7 agonist treatment (LPS or R848, respectively) for 24 h. Expression levels of MHC class I, CD80, and CD86 were evaluated by flow cytometry. LPS or R848 treatment strongly increased the expression of MHC class I on DCs. However, when these cells were co-treated with BTB-1, the expression level of MHC class I was significantly decreased (Fig. 4a, b). Similarly, LPS- or R848-induced expression of CD80 (Fig. 4c, d) and CD86 (Fig. 4e, f) was significantly decreased when treated with BTB-1. Although BTB-1 decreased expression levels of activation markers on unstimulated DCs (CTR) as well, its inhibitory effect was marginal as compared to that on LPS- or R848-stimulated DCs (Fig. 4). For example, BTB-1 decreased MFI for CD80 of unstimulated DCs less than 10%. However, BTB-1 decreased MFI for CD80 of LPS-stimulated DCs approximately 25% (Fig. 4c, d). Furthermore, since unstimulated (immature) dendritic cells are not efficient enough to induce the immune response, the inhibition by BTB-1 on unstimulated DCs would be immunologically neglectable. Therefore, our results support our

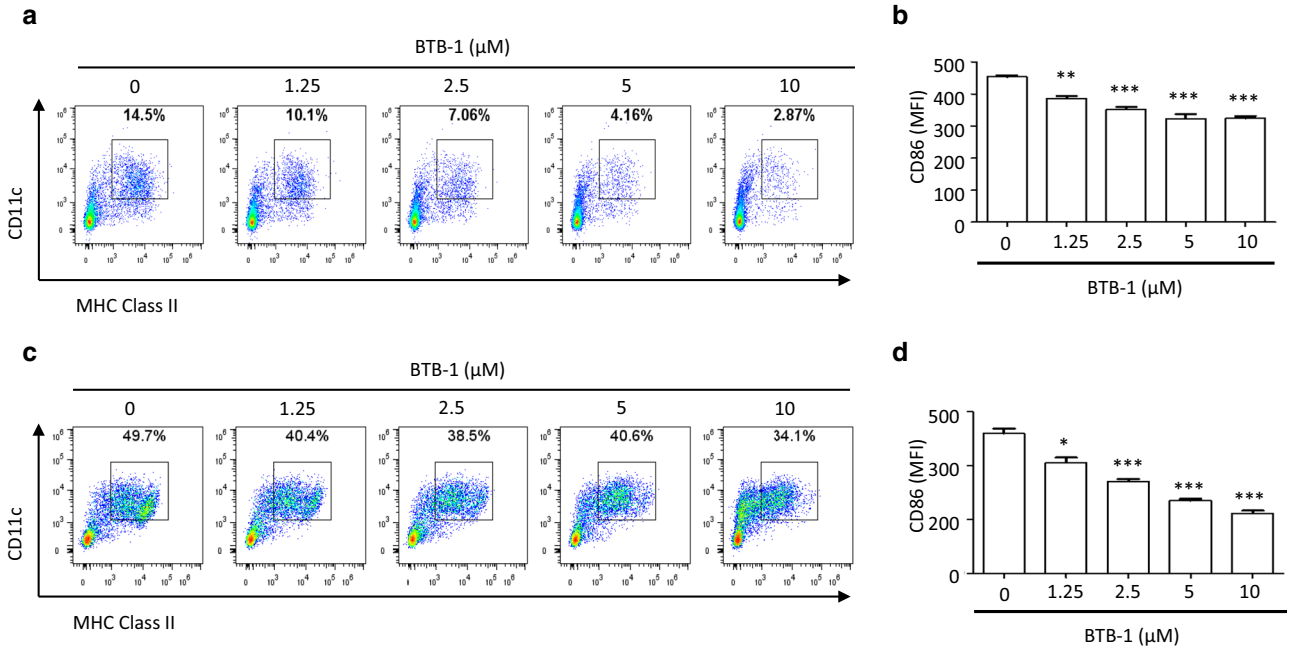

Fig. 3 Inhibition of KIF18A suppresses the differentiation of BM cells into DCs. Mouse BM cells were treated with BTB-1 at concentrations of 0, 1.25, 2.5, 5, and 10 μM. Three (**a** and **b**) or six (**c** and **d**) days later,frequency of CD11c$^+$/MHC II$^+$ cells (**a** and **c**) and the mean fluorescent intensity of CD86 (**c** and **d**) were analyzed by flow cytometry (**$P < 0.05$; ***$P < 0.001$)

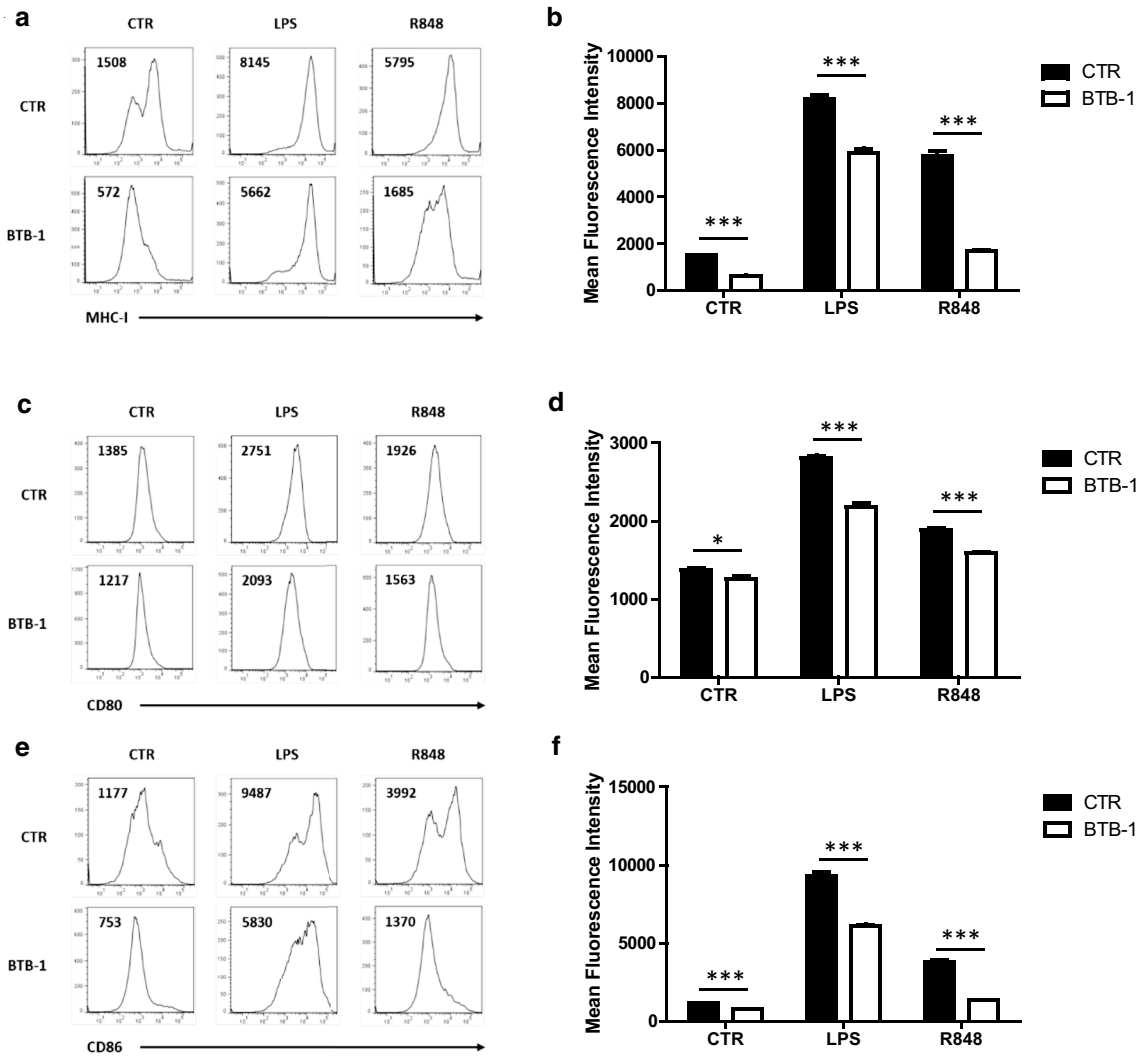

Fig. 4 KIF18A inhibition decreases the activation of DCs. BM-derived DCs were untreated or treated with BTB-1 (10 μM) upon stimulation with LPS (200 ng/mL) or R848 (1 ug/mL) for 24 h. The expression levels of MHC-I (**a** and **b**), CD80 (**c** and **d**), and CD86 (**e** and **f**) were analyzed by flow cytometry. The MFIs are shown along with a histogram for MHC-I (**a**), CD80 (**c**), and CD86 (**d**) (*$P<0.05$; ***$P<0.001$)

conclusion that BTB-1 suppressed activation of DCs upon TLR4 or TLR7 stimulation and thus, KIF18A could be an important target for immune-mediated diseases.

Discussion

DCs express diverse PRRs such as TLRs to recognize invading pathogens. For example, TLR4 binds to LPS, an outer membrane component of Gram-negative bacteria. TLR7 detects single-stranded RNAs from viruses such as influenza virus, HIV, and HCV (Kawai and Akira 2010). In this study, we found that the inhibition of KIF18A reduced the TLR4- and TLR7-induced activation of DCs. Since TLR4 and TLR7 share the MYD88 adaptor protein to initiate their intracellular signaling (Kawai and Akira 2006), it is possible that KIF18A is directly involved in trafficking signaling molecules down-stream of MYD88. Indeed, kinesins are shown to transport signaling molecules along microtubules (Yildiz and Selvin 2005). Thus, inhibition of KIF18A could also affect the transport of other molecules such as IL-1R and L-33R, which associate with the MYD88 adaptor protein.

Uncontrolled activation of TLR signaling often causes excessive immune responses, which can lead to severe immunopathology (Karlstrom et al. 2011; Lee et al. 2015). Thus, the inhibition of KIF18A could be beneficial in attenuating bacterial or viral infection-mediated immunopathology. Furthermore, since TLRs are expressed on DCs and other various immune cells including macrophages, neutrophils, mast cells, and B cells (Akira 2006), the function of

other immune cells expressing TLRs may also be impaired when treated with a KIF18A inhibitor. Testing this hypothesis will require further investigation.

In conclusion, the results of this study indicate that KIF18A is a critical host factor required for the differentiation and activation of DCs. Also, inhibition of KIF18A could be an efficient strategy for treating excessive immune response-mediated diseases.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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