Zinc Finger Protein 64 Promotes Toll-like Receptor-triggered Proinflammatory and Type I Interferon Production in Macrophages by Enhancing p65 Subunit Activation*3

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The molecular mechanisms that fine-tune the Toll-like receptor (TLR)-triggered innate immune response need further investigation. As an important transcription factor, zinc finger proteins (ZFPs) play important roles in many cell functions, including development, differentiation, tumorigenesis, and functions of the immune system. However, the role of ZFP members in the innate immune responses remains unclear. Here we showed that the expression of C2H2-type ZFP, ZFP64, was significantly up-regulated in macrophages upon stimulation with TLR ligands, including LPS, CpG oligodeoxynucleotides, or poly(I:C). ZFP64 overexpression promoted TLR-triggered TNF-α, IL-6, and IFN-β production in macrophages. Coincidently, knockdown of ZFP64 expression significantly inhibited the production of the above cytokines. However, activation of MAPK and IRF3 was not responsible for the ZFP64-mediated promotion of cytokine production. Interestingly, ZFP64 significantly up-regulated TLR-induced NF-κB activation. ZFP64 could bind to the promoter of the TNF-α, IL-6, and IFN-β genes in macrophages only after TLR ligation. Furthermore, ZFP64 associated with the NF-κB p65 subunit upon LPS stimulation, and TLR-ligated macrophages showed a lower level of p65 recruitment to the TNF-α, IL-6, and IFN-β gene promoter in the absence of ZFP64. The data identify ZFP64 as a downstream positive regulator of TLR-initiated innate immune responses by associating with the NF-κB p65 subunit, enhancing p65 recruitment to the target gene promoters and increasing p65 activation and, thus, leading to the promotion of TLR-triggered proinflammatory cytokine and type I interferon production. Our findings add mechanistic insight into the efficient activation of the TLR innate response against invading pathogens.

Toll-like receptors (TLRs)3 are crucial elements in sensing microbial infection for the induction of innate immune responses and the eventual elimination of invading microbial pathogens (1, 2). Upon recognition of TLR ligands, which can be pathogenic components referred to as pathogen-associated molecular patterns, TLRs activate a variety of proximal signaling pathways, such as the MyD88- and/or TRIF-dependent signaling cascades. These trigger several downstream events, including the activation of MAPK and nuclear transcription factors, in particular NF-κB, that, consequentially, induce the production of proinflammatory cytokines, type I interferon, and other response mediators, initiating the innate immune response and enhancing the adaptive immune response (3, 4).

Full activation of TLR signaling is essential for provoking potent anti-infectious immunity (5, 6). Less efficient activation of the TLR response may not elicit potent innate and adaptive immune responses against invading pathogens, leading to chronic infection. Therefore, deep insight into the molecular basis for efficient TLR activation is required for precise manipulation of the TLR response to prevent and treat infectious diseases. Recently, several molecules have been demonstrated to play critical roles in the activation of TLR signaling and innate immune response, such as Peli1 (7), Pin1 (8), Nrdp1 (9), CaMKII (10), and MHC class II (11). However, other unknown regulators and the underlying mechanisms for efficient activation of TLR signaling still remain to be further identified.

**Background:** The role of ZFPs in innate immune responses remains unclear.

**Results:** ZFP64 associates with the NF-κB p65 subunit, enhancing promoter recruitment and activation and, thus, promoting TLR-triggered proinflammatory cytokine and IFN-β production.

**Conclusion:** ZFP64 acts as a positive regulator of TLR signaling.

**Significance:** The data provide mechanistic insights into the efficient activation of the TLR response.

#References

1. The abbreviations used are: TLR, Toll-like receptor; ODN, oligodeoxynucleotides; TRIF, Toll/IL-1R domain-containing adapter-inducing IFN β; MyD88, myeloid differentiation factor 88; IRAK, interleukin-1 receptor-associated kinase; TRAF, TNF receptor-associated factor; CaMKII, calcium/calmodulin-dependent protein kinase II; ZFP, zinc finger protein; Q-PCR, quantitative PCR.

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Zinc finger protein (ZFP) is an important transcription factor family involved in a variety of cell functions (12). It is mainly divided into three types of finger proteins, the C2H2, C4, and C6 types, depending on the various structures and the highly conserved region. Among them, C2H2-type ZFP represents a classic and the largest kind of DNA binding transcription factors in the mammalian genome (13). Several studies have demonstrated that C2H2-type ZFP participates in the regulation of the host immune response. For instance, deficiency of ZDC in classic dendritic cells presented a higher inflammatory response to LPS (14). ZFP191 gene knockout mice were resistant to LPS-induced endotoxic shock (15). Recently, several studies with deficient mice of Gfi1, a C2H2 type transcription repressor, supported it as a negative regulator in immune response and inflammation (16–18). This indicates that C2H2-type ZFP may play a crucial role in innate immune response.

ZFP64 (also known as ZNF338) is a member of the Krüppel C2H2-type zinc finger family (19). Its biological function remains largely unknown, except for a report showing that it is a coactivator of Notch1, mediating mesenchymal cell differentiation (20). Interestingly, by RNA-seq analysis, we found that ZFP64 expression was up-regulated in mouse peritoneal macrophages in response to stimulation with the TLR4 ligand LPS with a 2-fold increase. In addition, a set of gene profiling data from the GEO microarray also revealed the inducible expression of ZFP64 in macrophages in response to stimulation with LPS (GEO accession no. GDS1285, 2003), CpG (GEO accession no. GDS1315, 2004), or active virus compared with inactive virus (GEO accession no. GDS3549, 2004). All of these data suggest that ZFP64 might be involved in the TLR-triggered innate immune response.

In this report, we addressed the function of ZFP64 in the TLR-initiated innate response. ZFP64 acts downstream of the TLR-triggered pathway by associating with the NF-κB subunit p65 and enhancing p65 recruitment to the promoters of its target genes and then its activation, thus leading to the promotion of TLR-triggered proinflammatory cytokine and type I interferon production. Therefore, our work revealed ZFP64 as a positive regulator in TLR signaling, providing new mechanistic insight into the efficient activation of the TLR innate response.

**EXPERIMENTAL PROCEDURES**

**Mice and Reagents—**C57BL/6 mice (six to 8 weeks) were obtained from Joint Ventures Sipper BK Experimental Animal Co., Shanghai, China. LPS (0111:B4) and poly(I:C) were from Sigma-Aldrich. Encoding HA-tagged ZFP64 was constructed by PCR cloning into the pEZ-M06 eukaryotic expression vector (Fulengine Genecopoeia). The MyD88 and TRIF expression vectors and NF-κB, TNF-α, IL-6, and IFN-β luciferase reporter plasmids were described previously (10). The FLAG-tagged p65 expression vector was constructed by PCR cloning into the pCDNA3.1 eukaryotic expression vector (Invitrogen). All constructs were confirmed by DNA sequencing.

**Real-time Quantitative PCR—**Total RNA was extracted using TRIzol regent (Invitrogen) and reverse-transcribed using a First Strand cDNA synthesis kit (Toyobo) following the instructions of the manufacturer. Real-time quantitative PCR (Q-PCR) analysis was performed with Light Cycler (Roche) and the SYBR RT-PCR kit (Takara). The primers used for ZFP64 were 5'-TGAAGGACGAGGATCAGACG-3' (sense) and 5'-TGCGAGAAAGGATCGTCCGT-3' (antisense). Data were normalized by the level of β-actin expression in each sample.

**Cell Culture and Transfection—**The mouse macrophage cell line RAW264.7 and the human HEK293T cell line from the ATCC were cultured and transfected with JetPEI (Polyplus). Thioglycolate-elicited mouse primary peritoneal macrophages were isolated and cultured as described previously (11).

**RNA Interference—**The specific siRNA for ZFP64 (si-ZFP64) and control siRNA (si-Non) were purchased from Santa Cruz and delivered into mouse peritoneal macrophages using INTERFERin reagent (Polyplus) as described previously (10). ELISA—TNF-α, IL-6, IFN-β, IL-10, and IL-12 levels in the supernatants were measured by ELISA (R&D Systems) according to the protocols of the manufacturer.

**Immunoblot and Immunoprecipitation Analysis—**Cells were lysed with cell lysis buffer (Cell Signaling Technology), and extracted protein was measured by BCA protein assay kit (Pierce). Immunoblot and immunoprecipitation analysis were performed with the indicated antibodies as described previously (21).

**Flow Cytometry Analysis for TLR Expression—**Expression of TLR3, TLR4, and TLR9 in mouse peritoneal macrophages was determined by FACS analysis. Briefly, cells were fixed with BD Cytofix/Cytoperm buffer (BD Biosciences) for 10 min, blocked with 1% FBS (resuspended in PBS) for 1 h, and then incubated for 30 min with anti-TLR3, anti-TLR4, or anti-TLR9 antibody, respectively. Cells were washed with PBS and analyzed using an LSR II flow cytometer (BD Biosciences). Proper isotype controls were included for TLR antibodies (22).

**Luciferase Reporter Gene Assay—**HEK293 cells were cotransfected with a mixture of the indicated luciferase reporter plasmid, pRL-TK-Renilla-luciferase plasmid, and the appropriate additional constructs for 24 h. Total amounts of DNA were equalized with empty control vector. Luciferase activities were measured using Dual-Luciferase reporter assay system (Promega) according to the instructions of the manufacturer. Data were normalized for transfection efficiency by comparing firefly luciferase activity with that of Renilla luciferase.

**ChIP assay—**Chromatin was immunoprecipitated according to the instructions of the manufacturer of the EZ ChIP kit (Millipore), with the following modifications. 1 x 10^7 cells were treated with LPS, poly(I:C), or CpG ODN, respectively, for 2 h, fixed for 20 min at 37 °C with 1% formaldehyde, and then lysed using SDS lysis buffer. Immunoprecipitation of chromatin
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RESULTS

TLR Signals Up-regulate ZFP64 Expression in Macrophages—As detected by our RNA-seq results as well as gene profiling data, an inducible expression of ZFP64 was shown by stimulation with TLR ligands, which inspired us to investigate the role of ZFP64 in the innate immune response. We firstly examined the expression pattern of ZFP64 in immune organs. ZFP64 was ubiquitously expressed in mouse normal tissues, including immune organs such as the thymus, bone marrow, and spleen (Fig. 1A). Among freshly isolated hematopoietic cells, ZFP64 was highly expressed in mouse peritoneal macrophages, B cells, and natural killer cells (Fig. 1B). Then we detected the effect of TLR signals on ZFP64 expression in macrophages. Stimulation with TLR ligands, including LPS, CpG ODN, and poly(I:C), significantly up-regulated ZFP64 mRNA expression in primary peritoneal macrophages, with a peak at 4 h after stimulation (Fig. 1C), which confirmed our RNA-seq result and gene profiling data. However, ZFP64 expression has hardly any effect on TLR expression because the expression of TLR3, TLR4, and TLR9 in primary peritoneal macrophages was barely changed by knockdown of ZFP64 expression by specific siRNA (supplemental Fig. S1).

ZFP64 Promotes the Production of Proinflammatory Cytokines and IFN-β in TLR-triggered Macrophages—Considering the TLR-induced up-regulation of ZFP64 expression in macrophages, we then evaluated the effect of ZFP64 on TLR-triggered cytokine production in macrophages. Overexpression of ZFP64 significantly increased LPS, CpG ODN, or poly(I:C)-induced production of proinflammatory cytokines such as TNF-α and IL-6 and also type I interferon, IFN-β (Fig. 2A). Consistently, silencing of ZFP64 expression significantly decreased LPS-, CpG ODN-, or poly(I:C)-induced production of TNF-α, IL-6, and IFN-β in mouse peritoneal macrophages (Fig. 2B). Our further experiments showed that cotransfection of ZFP64 could markedly enhance MyD88-activated TNF-α and IL-6 as well as TRIF-activated IFN-β reporter gene expression in a dose-dependent manner (Fig. 2C). These data provide convincing evidence that ZFP64 promotes both MyD88-dependent and TRIF-dependent proinflammatory cytokine and IFN-β production in macrophages. However, no substantial effect on TLR-triggered anti-inflammatory cytokine IL-10 and IL-12 production by ZFP64 silencing was observed (supplemental Fig. S2), suggesting that ZFP64 selectively promotes proinflammatory cytokine and IFN-β production in the TLR-triggered inflammatory response.

ZFP64 Enhances TLR-triggered NF-κB Activation—To elucidate the underlying mechanism for ZFP64 to promote the TLR-triggered immune response, we next examined the effect of ZFP64 on TLR-triggered signals, including proximal kinases (such as TAK1, TBK1, and IRAK4) and signaling pathways (such as MAPK and NF-κB). Silencing of ZFP64 expression in primary peritoneal macrophages had hardly any effect on the phosphorylation of TAK1, TBK1, and IRAK4, whereas silencing of ZFP64 significantly suppressed TLR-triggered activation of ERK1/2, JNK1/2, and p38. However, treatment with PD98059, a specific ERK inhibitor, had no effect on ZFP64-promoted LPS-triggered TNF-α and IL-6 production (Fig. 3C). These results suggest that ZFP64-mediated promotion of proinflammatory cytokine production may not be dependent on MAPK activation.

We further assessed the effect of ZFP64 on NF-κB and IRF3 signaling. ZFP64 knockdown did not alter the phosphorylation levels of IκBα or RelA/p65 that were crucial for controlling the transactivation potential of NF-κB (Fig. 4, A and B). In addition, we did not detect a deviation of the phosphorylation level of IRF3, whose activation contributes to TLR-induced transcriptional activation of the IFN-β gene (24, 25). Hence, it is likely that the signaling components of the NF-κB and IRF3 pathways...
in the TLR-triggered inflammatory response are not strongly affected by ZFP64.

Next, we explored the effect of ZFP64 on NF-κB and IRF3 activation. Cotransfection of ZFP64 with MyD88 significantly enhanced the reporter activity of NF-κB in a dose-dependent manner in HEK293T cells (Fig. 4C). NF-κB reporter activity was also promoted significantly by cotransfection of ZFP64 with TRIF, which could associate with TRAF6 and activate the NF-κB signal in TLR-triggered signaling. These results suggest that ZFP64 enhances both MyD88- and TRIF-mediated NF-κB activation. However, we observed hardly any effect of ZFP64 knockdown on the activation of IRF3 (Fig. 4D). Together with
the result that ZFP64 silencing did not affect TLR-triggered IRF3 phosphorylation (Fig. 4, A and B), our data excluded the possibility that IRF3 activation contributed to the promoting effect of ZFP64 on IFN-β production in the TLR-triggered response. Moreover, ZFP64 did not affect IL-1β- or TNF-α-induced NF-κB reporter activity (supplemental Fig. S3), which indicated that ZFP64 selectively promoted NF-κB activation in TLR signaling. Collectively, the above findings suggest that...
ZFP64 mediated promotion of TNF-α, IL-6, and IFN-β production by enhancing TLR-triggered NF-κB transcriptional activation.

**ZFP64 Binds to the Gene Promoters of TNF-α, IL-6, and IFN-β—** As a member of the ZFP family, ZFP64 may have a potential capacity of binding DNA and regulating gene transcription. We wondered whether ZFP64 could bind to the promoters of the TNF-α, IL-6, or IFN-β gene, leading to its gene activation and expression. A ChIP assay was performed with ZFP64 antibody in TLR-triggered macrophages. As shown in Fig. 5A, binding of ZFP64 to the gene promoters of TNF-α, IL-6, or IFN-β could hardly be observed in untreated macrophages. However, significant binding to these promoters was detected in LPS-, CpG ODN-, or poly(I:C)-stimulated macrophages (Fig. 5), suggesting that ZFP64 may bind to the promoters, promoting gene activation and resulting in the production of these cytokines in the TLR-triggered immune response.

**ZFP64 Associates with p65 and Enhances p65 Activation—** Given that ZFP64 could bind to the promoters of the TNF-α, IL-6, and IFN-β genes and enhance gene activation in macrophages only after TLR ligation (Fig. 2), we speculated that there are some intermediary factors involved in the process. Because ZFP64 promotes TLR-triggered NF-κB transcriptional activation (Fig. 4C), we then focused on p65 NF-κB subunit, which is a strong transcription factor for a wide variety of genes. To this end, we performed immunoprecipitation assays with extracts from HEK293T cells that cotransfected with the HA-tagged ZFP64 and FLAG-tagged p65 expression vectors. The result showed that p65 could be detected in ZFP64 immunoprecipitates (Fig. 6A) and that, similarly, ZFP64 could also be found in p65 immunoprecipitates (B). The association was further confirmed in macrophages that express both proteins endogenously. As shown in Fig. 6C, a slight association of ZFP64 with p65 could be detected in unstimulated macrophages, and the interaction was increased significantly after LPS stimulation for 15 and 30 min. An irrelevant control antibody (IgG) failed to coprecipitate the p65 subunit, excluding immunoprecipitation because of nonspecific binding. However, TNF-α stimulation could not significantly increase ZFP64 and p65 interaction in macrophages (supplemental Fig. S4). Furthermore, we found that p65 acetylation (on lysine 221, a main acetylation site that increases the DNA-binding affinity of p65 for κB sites) was needed for the interaction (supplemental Fig. S5). These data suggest that ZFP64 forms a complex with the NF-κB p65 subunit in the TLR-triggered innate response.

We further investigated which function of p65 was affected by the association with ZFP64. We found that ZFP64 could promote p65-mediated gene activation of TNF-α, IL-6, and IFN-β (Fig. 7A). Then, a ChIP assay was performed to evaluate the effect of ZFP64 on p65 recruitment to the target gene promoters. As shown in Fig. 7B, LPS-induced p65 binding to the gene promoter of TNF-α, IL-6, and IFN-β was decreased significantly by knockdown of ZFP64 expression, suggesting that ZFP64 enhances TLR-triggered p65 recruitment to its target gene promoters. Therefore, these results indicate that ZFP64 associates with p65 and promotes p65 transcription activity by enhancing TLR-triggered p65 recruitment to its target gene promoters, leading to the promoting effect on TLR-triggered proinflammatory cytokine and type I interferon production.
DISCUSSION

The role of ZFPs in the innate immune response has been a hot topic recently. In most cases, ZFPs were reported to be negative regulators in controlling the inflammation response with a distinct mechanism. The zinc finger protein tristetraprolin interacts with and destabilizes CCL3 mRNA, abrogating tissue-localized inflammatory processes (26). The CCCH zinc finger protein family members, including Zc3h12a, Zc3h12b, Zc3h12c, and Zc3h12d, suppressed LPS-triggered inflammatory cytokine production by inhibiting cytokine gene activation (27–29). In addition, Gfi1 (growth factor-independent 1) was found to antagonize inflammatory cytokine production and the TLR-triggered inflammation response (16–18). In contrast to most ZFPs known as negative regulators of TLR signaling, here we present evidence that the C2H2-type ZFP, ZFP64, promotes TLR-triggered proinflammatory cytokine and type I interferon production in macrophages by associating with the NF-κB subunit and enhancing TLR-triggered p65 activation. Thus, our data identify a unique mechanism utilized by ZFP that sustains the TLR-triggered innate immune response. Because full activation of signaling is essential for provoking a potent innate response as well as enhancing adaptive immunity against invading pathogens, our findings may have important implications and facilitate the development of new therapeutic strategies for the control of infectious diseases.

TLR activation is important for the initiation of the protective immune response, serving as the first line of host defense against infection. A number of regulatory mechanisms that can either promote or dampen TLR signaling pathways exist and have been reported, suggesting multiple distinct types of safety mechanisms for controlling invading pathogen infection as well as harmful inflammatory responses (30). Many of the regulators affect largely the expression, phosphorylation, or ubiquitination level of proximal signaling components, such as upstream adaptors, kinases, or ubiquitin ligases (e.g. MyD88, IRAK, TRIF, and TRAF). For example, Nrdp1 mediates MyD88 and TBK1 ubiquitination (9), CaMKII directly binds and activates TAK1 and IRF3 (10), intracellular MHC II molecules interact with the tyrosine kinase Btk (11), and constitutive membrane MHC I molecules physiologically interact with the tyrosine kinase Fps (22). The influence on the proximal part of TLR signaling then impacts more distal post-TLR signaling events, such as the activation of MAPKs and transcription factors (e.g. NF-κB, AP-1, or IRF3). Our results suggest that ZFP64 hardly affects TLR signaling at the proximal level, including TAK1, TBK1, IRAK4, and signaling components of NF-κB (1xβTr and p65), and IRF3 activation (Fig. 4, A and B). Although increased activation of TLR-triggered cytoplasmic signaling molecules of the MAPK pathway by ZFP64 was observed, it is not required for ZFP64-mediated promotion of cytokine production (Fig. 3). Instead, ZFP64 acts at the downstream end of the TLR-triggered pathway. By associating with p65 and enhancing its recruitment to the promoters of its target genes and then its activation. Our data, as well as the data of others (17, 31), have revealed that
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regulation of NF-κB activity is not necessarily caused via the engagement of proximal TLR signaling and may be regulated by nuclear activators or repressors.

NF-κB signaling is a major signaling pathway activated when cells are challenged with a variety of stimuli, such as inflammatory stimuli and pathogen-associated molecular patterns (32, 33). Upon infection with a pathogen or stimulation with a TLR ligand, NF-κB signaling is activated via the phosphorylation and degradation of IkBα through the IkB kinase complex, which leads to the nuclear translocation of NF-κB (34). The NF-κB subunit p65 binds to the promoter of NF-κB-related genes such as TNF-α, IL-6, and IFN-β and induces gene transcription (35). Thus, the regulation of NF-κB signaling activation is crucial for the proper induction of TLR-mediated inflammatory responses. However, only a handful of studies so far show that zinc finger proteins regulate the TLR-triggered immune response by targeting the NF-κB signaling pathway. For example, Gfi1 attenuates the TLR4-mediated inflammatory response by antagonizing NF-κB transcription activity (16, 17). Our study demonstrated that ZFP64 could positively regulate the TLR-induced inflammatory response by promoting p65 transcriptional activation. This effect could be attained through two mechanisms: by directly enhancing p65 action, e.g., its binding to DNA, and by promoting its transactivation capacity. Our data support the first mechanism. Several lines of evidence support this hypothesis. First, immunoprecipitation assays revealed that both exogenously and endogenously expressed ZFP could interact with p65 (Fig. 6). Secondly, reporter gene assays showed that ZFP64 promotes NF-κB p65-dependent transcription of synthetic target gene promoters appended to a luciferase gene (Fig. 7A). Thirdly, ChIP assays with primers covering TNF-α, IL-6, and IFN-β promoter demonstrated a significantly lower rate of occupancy of the proximal NF-κB binding site by p65 in ZFP64 knockdown macrophages than in control cells (Fig. 7B). Last, mutation of lysine 221 (a main acetylation site that increases the DNA-binding affinity of p65 for κB sites) (36) of p65 impaired the interaction of ZFP64 and p65 (supplemental Fig. S5). Thus, we propose here a model in which ZFP64 interacts with p65 and enhances its binding to its cognate binding sites present in the TNF-α, IL-6, and IFN-β promoters and, therefore, its transcriptional activation.

It remains to be seen how ZFP64 interaction with p65 enhances the DNA binding capacity of p65. One possibility is that ZFP64 directly binds to cytokine gene promoters and simply “attracts” or “drags” p65 to its binding sites. In this case, less p65 is able to access the target gene promoters in the absence of ZFP64, as reflected by lower p65 recruitment in the ChIP assays (Fig. 7B). However, reporter gene assays showed that ZFP64 itself (without cotransfection with the MyD88 or TRIF expression vector) could not enhance TNF-α, IL-6, and IFN-β gene activation (Fig. 2C), and ChIP assays showed that ZFP64 could hardly bind to the cytokine gene promoters in TLR-unligated macrophages (Fig. 5). These data basically exclude the possibility that ZFP64 occupies gene promoters in a direct way that does not favor the abovementioned possibility. Alternatively, ZFP64, as a zinc-finger transcription factor family member, may also have a potential capacity of regulating gene transcription, probably by activating a “helper molecule” of p65. This seems very likely in light of our findings reported here as well as the known function of ZFP64 as a coactivator of Notch1 implicated to be involved in TLR-triggered immune response (37–39). We also found that p65 acetylation was needed for the interaction. Serial studies have demonstrated that multiple molecules could affect p65 acetylation in response to specific inflammatory stimuli, allowing a distinct acetylation pattern for p65 (40, 41), which could be one possible explanation for the selective association of ZFP64 with TLR-induced NF-κB activation. Therefore, it needs to be investigated whether ZFP64 binds to other intermediary factors (e.g. Notch1), which enhances NF-κB activation and the TLR-triggered immune response.

Taken together, we identify ZFP64 as a positive regulator for TLR signaling, which is critical for innate inflammatory signal transduction after the host immune system recognizes the invading pathogens. Our results suggest that ZFP64 promotes the activation of the TLR signaling pathway by enhancing NF-κB activation at the downstream end point of TLR signaling in the nucleus. This provide new insights into the mechanisms that can lead to the efficient activation of the TLR innate response and may provide a theoretical basis for future interventional strategies to control infectious diseases.

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