HHS Public Access

Author manuscript

Nat Immunol. Author manuscript; available in PMC 2015 December 01.

Published in final edited form as:

Nat Immunol. 2015 June ; 16(6): 642–652. doi:10.1038/ni.3155.

SHP-2 Mediates C-type Lectin Receptors-induced Syk Activation and Anti-fungal T_H17 Responses

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SUMMARY

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Fungal infection stimulates the canonical C-type lectin receptors (CLRs) signaling pathway via Syk activation. Here we show that SHP-2 plays a crucial role in mediating CLRs-induced Syk activation. Genetic ablation of *Shp-2* (*Ptpn11*) in dendritic cells (DCs) and macrophages impaired Syk-mediated signaling and abrogated pro-inflammatory gene expression following fungal stimulation. Mechanistically, SHP-2 operates as a scaffold facilitating the recruitment of Syk to dectin-1 or FcRγ, through its N-SH2 domain and a previously unrecognized C-terminal ITAM motif. We demonstrate that DC-derived SHP-2 is crucial for the induction of IL-1β, IL-6 and IL-23, and anti-fungal T_H17 cell responses to control *Candida albicans* infection. Together, these data reveal a mechanism by which SHP-2 mediates Syk activation in response to fungal infections

Keywords

C-type lectin receptors; SHP-2; *Candida albicans*; T_H17

Candida albicans is a serious health threat worldwide, particularly among the elderly and immunocompromised individuals[1,2]. Despite being a normal commensal of healthy individuals, *C. albicans* can become highly pathogenic and cause severe mucosal and systemic candidiasis in AIDS, organ-transplanted and immune-deficient patients[3]. Current anti-fungal therapy is often ineffective and may cause undesirable side effects. Therefore a better understanding of the host defense mechanisms may lead to the development of effective preventive and therapeutic strategies[2,4].

Recent studies have begun to uncover the fundamental mechanisms of innate and adaptive immune responses against fungal infections. A number of innate receptors, such as TLRs, C-type lectin receptors and NLRs, have pivotal roles in host defense against fungal pathogens by sensing their pathogen-associated pattern molecules[5,6]. C-type lectin receptors dectin-1, dectin-2, dectin-3 and Mincle detect β-glucan[7], α-mannan[8] or glycolipid[9], and thereby initiate innate and adaptive immune responses to pathogenic fungi. Dectin-1, the dectin-2-dectin-3 (Dectin-2/3) heterodimer and Mincle can initiate complex signaling pathways, inducing the production of myriad cytokines and chemokines (including IL-1-β, IL-12, IL-6, IL-23, IFN-β, TNF, CXCL-1 and CXCL-2)[8,10]. Collectively, CLRs-induced pro-inflammatory chemokines and cytokines can trigger neutrophil influx, macrophage maturation[11,12], and T cell differentiation[3,13,14]. Whereas T_H1 cells have been implicated in fungal infection[2], T_H17 cells are the major T cell subset responsible for eliminating fungal pathogens, primarily by secreting cytokines IL-17A and IL-17F[6,14]. Consistent with this notion, humans deficient for IL-17A or IL-17R have the propensity to develop mucosal candidiasis[15]. Although dectin-1 and dectin-2/3 are widely expressed in neutrophils, macrophages, monocytes and dendritic cells (DCs), it remains unclear how they orchestrate host defense in these cellular compartments[16].

Following stimulation by their respective ligands, CLRs induce activation of NF-κB, MAPKs and NFATs[17], as well as Caspase-1/8[18], which in turn induce pro-inflammatory cytokines and chemokines. Canonical CLR signaling begins with the activation of spleen tyrosine kinase (Syk), which leads to NF-κB and MAPKs activation. Once recruited to the C-type lectin receptor complexes, Syk becomes phosphorylated and activated, primarily

through an inter-molecular autophosphorylation mechanism. Activated Syk then promotes the phosphorylation of downstream signaling molecules phospholipase PLCγ2[19,20] and PKCδ[21], which phosphorylates CARD9, resulting in the assembly of CARD9-Bcl10-Malt1 complex. The CARD9-Bcl10-Malt1 complex is responsible for activation of the canonical TAK1-IKKα/β-IκBα/p65 pathway[22]. Syk contains tandem N-SH2 and C-SH2 domains at its N-terminus, followed by a C-terminal kinase domain. Structural and biochemical analyses suggest the SH2 domains must bind to the phosphor-YXXI/L sequences within an ITAM motif (YXXI/L$X_{6–12}$YXXI/L) to activate Syk[23]. After engagement by particulate β-glucan or *C. albicans*, dectin-1 forms clusters or "phagocytic synapses", leading to the activation of Src family kinases[24], which induces the phosphorylation of dectin-1 and leading to Syk activation[25]. Because the cytoplasmic tail of dectin-1 contains only one YXXI/L motif called HemITAM[7,25], it is unclear how dectin-1 engages both SH2 domains of Syk simultaneously. In contrast, dectin-2/3 and Mincle do not have an ITAM motif but utilize ITAM-containing adaptor FcRγ to mediate Syk activation[26,27].

SHP-2, a ubiquitously expressed cytoplasmic tyrosine phosphatase that is involved in the signaling of growth factors, cytokines and hormones[28–31] is composed of two tandem SH2 domains, a PTP domain and a C-terminal tail containing phosphor-tyrosine residues[32,33]. Here, we report that a cryptic ITAM motif in the C-terminus of SHP-2 is phosphorylated upon ligation of dectin-1, dectin-2/3 or Mincle by β-glucan, mannan, TDB or *C. albicans*. Phosphorylated SHP-2 then functions as a scaffold protein recruiting Syk to dectin-1 or dectin-2/3 receptor complexes, and leading to the activation of Syk and Syk-dependent signaling events. Consistently, SHP-2 is required for the induction of pro-inflammatory cytokines and chemokines elicited by multiple C-type lectin receptors. Using *Shp-2*-deficient mice in myeloid cells, we provide genetic evidence supporting the concept that macrophages, neutrophils and DCs have distinct roles in the initiation of anti-fungal T_H17 responses.

RESULTS

Dectin-1 signaling induces SHP-2 activation

Protein tyrosine kinases including Src and Syk play a critical role in C-type lectin receptor signaling by initiating a cascade of tyrosine-phosphorylation events[21,24]. Bone-marrow derived DCs (BMDCs) differentiated by GM-CSF and IL-4 stimulated by the dectin-1 ligand Zymd, which is Zymosan depleted of TLR ligands, multiple tyrosine-phosphorylated bands were detected by anti-phosphor-tyrosine (p-Tyr) (Fig. 1a). Using a panel of phosphor-specific antibodies we identified the respective phosphorylated proteins (Fig. 1b and **data not shown**). Consistent with previous reports, p-Syk, p-PLCγ2 and p-PKCδ were induced following Zymd stimulation (Fig. 1b). Interestingly, we also found that p-SHP-2 (at Y_{542}) was highly induced in BMDCs upon dectin-1 engagement with Zymd (Fig. 1b). To validate this, we examined tyrosine-phosphorylation of SHP-2 in bone-marrow derived macrophages (BMDMs) primed by IL-4 and found that p-SHP-2 (at Y_{542}) was also markedly induced in BMDMs stimulated by Zymd (Supplementary Fig. 1a). We also performed immunoprecipitation using anti-p-Tyr or anti-SHP-2, followed by anti-SHP-2 or anti-p-Tyr blotting, respectively, and confirmed that SHP-2 was phosphorylated after Zymd stimulation

(Fig. 1c). To identify the kinase responsible for phosphorylation of SHP-2 following dectin-1 stimulation, we added Src or Syk inhibitors, and found they blocked SHP-2 phosphorylation (Fig. 1d). To determine the role of dectin-1 in Zymd-induced responses, we blocked dectin-1 responses using laminarin or utilized dectin-1-deficient BMDCs. We found phosphorylation of SHP-2 and Syk was abrogated in laminarin-treated wild-type BMDCs and dectin-1-deficient BMDCs (Supplementary Fig. 1b, c), indicating that SHP-2 phosphorylation is induced by dectin-1, and not by TLR2 signaling. Moreover, Zymd-induced TNF production was also abolished in dectin-1-deficient BMDCs (Supplementary Fig. 1d). These results collectively suggest that dectin-1 signaling triggers SHP-2 phosphorylation at Tyr542, possibly by Src and/or Syk kinases. It is important to note that macrophages and BMDCs from wild-type mice responded poorly to Zymd stimulation (Supplementary Fig. 1e–g), and IL-4 priming considerably elevated dectin-1 signaling in wild-type BMDMs and BMDCs (Supplementary Fig. 1e,f), but decreased TLR signaling in wild-type BMDCs (Supplementary Fig. 1g), we therefore used BMDMs primed by IL-4 and BMDCs differentiated by GM-CSF and IL-4 from all the mouse strains for dectin-1 ligand stimulation in this study.

To examine the role of SHP-2 in dectin-1 signaling, we bred *Shp-2* floxed mice with a *Cd11c*-cre transgenic strain to generate *Cd11c*-cre:*Shp-2*$^{fl/fl}$ mice that are deficient for *Shp-2* in DCs (designated as DC-*Shp-2*$^{-/-}$ mice). The deletion efficiency of *Shp-2* in BMDCs from DC-*Shp-2*$^{-/-}$ mice was over 80%, and *Shp-2* was not deleted in macrophages, T cells or B cells isolated from DC-*Shp-2*$^{-/-}$ mice (Supplementary Fig. 1h). Moreover, expression of dectin-1, dectin-2, dectin-3, Mincle and FcRγ were comparable in control and DC-*Shp-2*$^{-/-}$ BMDCs (Supplementary Fig. 1i). BMDCs from *Shp-2*$^{fl/fl}$ and DC-*Shp-2*$^{-/-}$ mice were stimulated by dectin-1 ligands (Zymd, ZymA (Zymosan A) and Curdlan), and cytokine and chemokine production was measured. Strikingly, all the chemokines (CXCL1 and CXCL2) and cytokines (TNF, IL-1β, IL-6, IL-12 and IL-23) induced by three different dectin-1 ligands were significantly diminished in DC-*Shp-2*$^{-/-}$ BMDCs (Fig. 1e). Next, we bred *Shp-2* floxed mice with a *LysM*-cre transgenic strain to generate *LysM*-cre:*Shp-2*$^{fl/fl}$ mice that are deficient for *Shp-2* in macrophages and neutrophils (designated as M/N-*Shp-2*$^{-/-}$ mice). The deletion efficiency of *Shp-2* in BMDMs from M/N-*Shp-2*$^{-/-}$ mice was over 80%, *Shp-2* was not deleted in DCs, T cells or B cells isolated from M/N-*Shp-2*$^{-/-}$ mice (Supplementary Fig. 1h) and expression of dectin-1, dectin-2, dectin-3, Mincle and FcRγ were comparable in BMDMs from *Shp-2*$^{fl/fl}$ and M/N-*Shp-2*$^{-/-}$ mice (Supplementary Fig. 1j). Deletion of *Shp-2* in BMDMs from M/N-*Shp-2*$^{-/-}$ mice also abrogated pro-inflammatory chemokine and cytokine production elicited by dectin-1 ligands (Supplementary Fig. 2a). Since SHP-2 has been previously implicated in TLR4 signaling[34], we also examined TLR-induced pro-inflammatory response in DC-*Shp-2*$^{-/-}$ BMDCs. Unlike Zymd stimulation which was decreased in DC-*Shp-2*$^{-/-}$ BMDCs, the TLR4 ligand LPS induced more TNF production in DC-*Shp-2*$^{-/-}$ BMDCs, whereas other TLR ligands Pam3 (TLR2), poly I:C (TLR3), and CpGB (TLR9), and NOD2 ligand MDP triggered similar concentrations of TNF in DC-*Shp-2*$^{-/-}$ BMDCs (Supplementary Fig. 2b). Taken together, these experiments identified SHP-2 as a positive regulator of dectin-1 signaling.

SHP-2 mediates anti-fungal-induced innate immunity

Dectin-1, along with dectin-2/3, mediates *C. albicans*-induced gene expression by recognizing the fungal cell wall components β-glucan and α-mannan, respectively. Next, we examined the role of SHP-2 in mediating *C. albicans*-induced pro-inflammatory gene expression. BMDCs from control and DC-*Shp-2*$^{-/-}$ mice were stimulated with heat-killed *C. albicans* yeast or hyphae, and cytokine production measured. DC-*Shp-2*$^{-/-}$ BMDCs produced significantly less chemokines (CXCL1 and CXCL2) and cytokines (TNF, IL-1 β, IL-6, IL-12 and IL-23) compared to control BMDCs after stimulation with either *C. albicans* yeast or hyphae (Fig. 2a), indicating that SHP-2 has an important role in regulating the innate immune response to pathogenic fungi.

To determine if there is a role for SHP-2 in C-type lectin signals other than dectin-1, we stimulated wild-type, FcRγ-deficient and Mincle-deficient BMDCs with mannan and Trehalose-6, 6-dibehenate (TDB), which are ligands for dectin-2/3 and Mincle, respectively. Mannan-induced Syk phosphorylation and TNF production were abolished in FcRγ-deficient and TDB-induced Syk phosphorylation and TNF production were abolished in Mincle-deficient BMDCs, indicating their specific engagement to dectin-2/3 or Mincle, respectively (Fig. 2b, Supplementary Fig. 2c). Similar to Zymd, mannan and TDB induced SHP-2 phosphorylation in FcRγ- or Mincle-dependent manner in BMDCs, indicating that SHP-2 phosphorylation is also induced by dectin-2/3 and Mincle signaling (Fig. 2b). We examined mannan- and TDB-induced pro-inflammatory gene expression in wild-type and DC-*Shp-2*$^{-/-}$ BMDCs, and found that DC-*Shp-2*$^{-/-}$ BMDCs produced significantly less CXCL1, CXCL2 and TNF than wild-type BMDCs after stimulation with these ligands (Fig. 2c,d). Moreover, chemokine and cytokine production was also attenuated in M/N-*Shp-2*$^{-/-}$ BMDMs stimulated with *C. albicans*, mannan, or TDB (Supplementary Fig. 2d–f). Collectively, these results demonstrated that SHP-2 participates in multiple C-type lectin receptor signaling pathways and plays a critical role in innate immunity to fungal pathogens.

SHP-2 mediates Syk activation induced by *C. albicans*

Next, we dissected the role of SHP-2 in regulating dectin-1 and *C. albicans*-induced signaling. Heat-killed *C. albicans* yeast mainly engages dectin-1, whereas the hyphae form primarily engages dectin-2[20]. We therefore used HKCA yeast to stimulate dectin-1 signaling in BMDCs from wild-type and DC-*Shp-2*$^{-/-}$ mice. Phosphorylation of Syk induced by Zymd or *C. albicans* was decreased in DC-*Shp-2*$^{-/-}$ BMDCs compared to wild-type BMDCs (Fig. 3a,b). Further, Syk-dependent phosphorylation of PLCγ2 and PKCδ was abrogated in DC-*Shp-2*$^{-/-}$ BMDCs. PKCδ plays an essential role in dectin-1-induced IKKα/β activation, which mediates IκBα phosphorylation and NF-κB activation[21]. We also observed that Zymd- or *C. albicans*-induced p-IκBα was attenuated in DC-*Shp-2*$^{-/-}$ BMDCs (Fig. 3a,b), indicating that dectin-1-induced NF-κB activation is SHP-2-dependent. In addition, ERK and JNK phosphorylation was also diminished in DC-*Shp-2*$^{-/-}$ BMDCs stimulated by either Zymd or *C. albicans* (Fig. 3a,b). Raf-1 was also activated by dectin-1 and *C. albicans* stimulation[13], and its phosphorylation reduced in DC-*Shp-2*$^{-/-}$ BMDCs (Fig. 3a,b). Intriguingly, p38 phosphorylation was not impaired in DC-*Shp-2*$^{-/-}$ BMDCs (Fig. 3a,b), indicating that dectin-1 signaling may activate p38 through Syk- and Raf-1-independent pathway. We also examined HKCA hyphae-induced signaling events in wild-

type and DC-*Shp-2*$^{-/-}$ BMDCs, and obtained the same results as HKCA yeast stimulation (**Data not shown**). Furthermore, Syk activation and Syk-dependent signaling events were also impaired in M/N-*Shp-2*$^{-/-}$ BMDMs following Zymd or *C. albicans* stimulation (Supplementary Fig. 3a,b). Together, these data indicate that SHP-2 regulates Syk activation in dectin-1 and *C. albicans*-induced signaling.

Next, we investigated whether SHP-2 interacts with Syk in dectin-1 signaling. By anti-SHP-2 immunoprecipitation, we found that Zmyd induced SHP-2 interaction with Syk in wild-type BMDCs (Fig. 3c). Conversely, purified Syk proteins were able to efficiently pull-down SHP-2 proteins, which were tyrosine-phosphorylated in BMDCs following Zymd stimulation (Fig. 3d). These data demonstrate that SHP-2 interacts with Syk after the activation of dectin-1 signaling. We also examined whether SHP-2 interacts with other molecules involved in dectin-1 signaling. By co-immunoprecipitation, we found that SHP-2 associated with Src, but not with Raf-1, PLCγ2, PKCδ or CARD9 in Zymd-stimulated wild-type BMDCs (Fig. 3c). By biochemical fractionation and confocal microscopy, we observed that SHP-2 translocated to the cell membrane, where it colocalized with dectin-1 after Zymd stimulation (Fig. 3e,f). Similarly, Syk resided predominantly in the cytosol in unstimulated wild-type BMDCs, but following Zymd stimulation, Syk was recruited to the cell membrane and colocalized with SHP-2 (Fig. 3e,f). Both Zymd and mannan stimulation mobilized SHP-2 and Syk to the cell membrane of the wild-type BMDCs (Fig. 4a), where it colocalized with the dectin-1 or dectin-2/3 adaptor FcRγ (Fig. 4b,c). Zymd or mannan-induced Syk recruitment to the cell membrane and colocalization with dectin-1 or FcRγ were abrogated in DC-*Shp-2*$^{-/-}$ BMDCs (Fig. 4a–c). Taken together, these results suggest that SHP-2 mediates the recruitment of Syk to dectin-1 or dectin-2/3-FcRγ.

Because Syk is also involved in BCR- and FcγR-mediated signaling, we investigated whether SHP-2 is also required for Syk activation in B cells and macrophages. In splenic B cells, IgM stimulation induced the phosphorylation of SHP-2 and Syk but *Shp-2*$^{-/-}$ B cells from *Vav*-cre:*Shp-2* floxed mice exhibited reduced IgM-induced Syk phosphorylation (Supplementary Fig. 3c). Furthermore, cross-linking of FcγRIII using anti-CD16 induced Syk phosphorylation in wild-type BMDMs but not in M/N-*Shp-2*$^{-/-}$ BMDMs (Supplementary Fig. 3d). Syk phosphorylation induced by anti-OVA IgG and ovalbumin complexes, as well as aggregated IgG, was also diminished in M/N-*Shp-2*$^{-/-}$ BMDMs (Supplementary Fig. 3e,f). These findings extend the role of SHP-2 in Syk activation to a range of signaling receptors on B cells, macrophages and DCs.

SHP-2 binds to Syk through its C-ITAM motif

Next, we dissected the mechanisms by which SHP-2 regulates the recruitment and activation of Syk in dectin-1 and dectin-2/3 signaling. By exogenously expressing dectin-1 and Syk in HEK293T cells, we only detected modest phosphorylation and activation of Syk following stimulation with heat-killed *C. albicans*. However, simultaneous expression of SHP-2 greatly enhanced Syk activation (Supplementary Fig. 4a). Using co-immunoprecipitation, we found that dectin-1 and Syk formed complexes, which were promoted by exogenous SHP-2 and were greatly enhanced by the stimulation with heat-killed *C. albicans*

(Supplementary Fig. 4b). In this system, SHP-2 also functions as a scaffold protein facilitating Syk recruitment to dectin-1.

SHP-2 possesses two SH2 domains at the N-terminus (referred to N-SH2 and C-SH2 domain here), followed by a PTP domain and a phosphor-tyrosine-containing tail[32,35]. To determine the structural basis for its scaffolding function, we generated a series of SHP-2 mutants and tested their ability to interact with dectin-1. Deletion of the N-SH2 domain abolished the association of SHP-2 and dectin-1 (Fig. 5a). Furthermore, the N-SH2 of SHP-2 alone was able to strongly interact with dectin-1. Intriguingly, deletion of the C-SH2 of SHP-2 also diminished its association with dectin-1. However, C-SH2 domain alone did not interact with dectin-1, excluding its direct involvement in the SHP-2-dectin-1 association (Fig. 5a). Moreover, the $Y_{15}F$ mutant of dectin-1 failed to recruit SHP-2 upon *C. albicans* stimulation in HEK293T cells (Fig. 5b). These results indicate that SHP-2 is recruited to the dectin-1 hemITAM via its N-SH2 domain.

We next examined how SHP-2 recruits Syk in dectin-1 signaling. Upon *C. albicans* stimulation, SHP-2 interacted with the wild-type, but not the N-SH2 or C-SH2 deletion mutants of Syk (Fig. 5c), implicating a possible involvement of the ITAM motif and SH2 domains in the SHP-2-Syk interaction. Indeed, we identified a putative ITAM motif composed of **Y_{511}**RF**I**YMAVQHYIETLQRRIEEEQKSKRKGHE**Y_{542}**TN**I** at the C-terminus of SHP-2. Consistent with our data showing SHP-2 is phosphorylated at Y_{542}, mutating Y_{542} to F_{542} reduced the phosphorylation of SHP-2 elicited by *C. albicans* stimulation. Similarly, mutating Y_{511} to F_{511} also diminished SHP-2 phosphorylation following *C. albicans* stimulation (Fig. 5d), indicating that Y_{511} is also phosphorylated by dectin-1 and dectin-2/3 signaling. Furthermore, mutation of either Y_{542} or Y_{511} also abrogated the SHP-2 interaction with Syk (Fig. 5e). These results imply that this non-canonical ITAM motif at SHP-2 C-terminus is required for the binding to the SH2 domains of Syk.

To substantiate these findings, we generated a series of SHP-2 mutants and tested their ability to restore dectin-1 signaling in M/N-*Shp-2*$^{-/-}$ BMDMs. Following lentiviral transduction of the wild-type and mutants of SHP-2, we found the expression of the exogenous SHP-2 mutants were either similar to or more highly than the wild-type SHP-2 proteins (Fig. 5f). Reexpression of SHP-2 efficiently restored the induction of TNF by Zymd in M/N-*Shp-2*$^{-/-}$ BMDMs, however, the N-SH2 deletion mutant, or mutants of $Y_{542}F$ or $Y_{511}F$ failed to restore Zymd-induced TNF expression in M/N-*Shp-2*$^{-/-}$ BMDMs (Fig. 5f). Conversely, the phosphatase-inactive mutant of SHP-2 (SHP-2 PD ($C_{459}S$)) was still able to restore TNF induction in M/N-*Shp-2*$^{-/-}$ BMDMs (Fig. 5f). Like the wild-type SHP-2, the phosphatase-inactive mutant of SHP-2 efficiently recruited Syk to dectin-1 in HEK293T cells stimulated by heat-killed *C. albicans* (Supplementary Fig. 4c). Furthermore, the phosphatase-inactive mutant of SHP-2 was able to mediate the recruitment of Syk to dectin-1 and promote Syk phosphorylation in wild-type BMDMs stimulated by Zymd (Supplementary Fig. 4d–f). These results further support the hypothesis that the N-SH2 and C-terminal ITAM motif of SHP-2 play a crucial role in recruiting Syk to dectin-1 (Supplementary Fig. 5a).

Upon dectin-2/3 activation by mannan or *C. albicans*, SHP-2 also enhanced the recruitment of Syk to FcRγ in HEK293T cells (Supplementary Fig. 5b). Furthermore, we found that both N-SH2 and C-SH2 domains of SHP-2 were involved in the binging to FcRγ (Supplementary Fig. 5c), and mutation of either Y_{65} or Y_{77} within the ITAM motif of FcRγ abrogated SHP-2 binding following *C. albicans* stimulation (Supplementary Fig. 5d). These results indicate that SHP-2 uses both SH2 domains to bind the ITAM motif of FcRγ in dectin-2/3 signaling (Supplementary Fig. 5e).

DC-SHP-2 is required for anti-fungal T_H17 responses

To determine whether SHP-2-mediated dectin-1 and dectin-2/3 signaling in DCs regulates anti-fungal immune responses *in vivo*, mice were infected intravenously (i.v.) with *C. albicans*. We observed much higher morbidity and mortality in DC-*Shp-2*$^{-/-}$ mice compared with *Shp-2*$^{fl/fl}$ mice (Fig. 6a). While *Shp-2*$^{fl/fl}$ mice showed moderate weight loss, with 40% survival and recovery, DC-*Shp-2*$^{-/-}$ mice exhibited drastic weight loss after infection, and eventually succumbed to *C. albicans* infection (Fig. 6a). Compared to *Shp-2*$^{fl/fl}$ mice, DC-*Shp-2*$^{-/-}$ mice had much higher fungal loads in the kidney, liver and spleen (Fig. 6b). We also found increased *Candida* hyphae in DC-*Shp-2*$^{-/-}$ kidneys 5 days after infection (Supplementary Fig. 6a).

We further examined the innate and adaptive immune responses in infected *Shp-2*$^{fl/fl}$ and DC-*Shp-2*$^{-/-}$ mice. One day after infection, TNF, IL-6, IL-12, CXCL1 and CXCL2 were detected in the sera of *Shp-2*$^{fl/fl}$ mice (Fig. 6c). Notably, TNF, IL-6 and IL-12 induction, but not CXCL1 and CXCL2, were attenuated in DC-*Shp-2*$^{-/-}$ mice. The numbers of neutrophils and macrophages infiltrated into the kidneys of DC-*Shp-2*$^{-/-}$ mice were not significantly different from those in control kidneys 3 days after infection (Supplementary Fig. 6b). Five days after infection, IL-17A and IL-17F gene expression was elevated in the kidneys of infected *Shp-2*$^{fl/fl}$ mice, but not in the kidneys of DC-*Shp-2*$^{-/-}$ mice (Fig. 6d). Furthermore, IFN-γ production was also undetectable in the kidneys of DC-*Shp-2*$^{-/-}$ mice (Fig. 6d). Spleen cells were isolated from infected *Shp-2*$^{fl/fl}$ and DC-*Shp-2*$^{-/-}$ mice and stimulated *ex vivo* with heat-killed *C. albicans*, and IL-17A was measured. Consistent with the *in vivo* data, DC-*Shp-2*$^{-/-}$ mice exhibited a reduced anti-fungal-specific T_H17 response compared with *Shp-2*$^{fl/fl}$ mice (Fig. 6e). Moreover, T_H1 responses against *C. albicans* were also diminished in DC-*Shp-2*$^{-/-}$ mice (Fig. 6e). These results demonstrate that DCs rely on SHP-2-mediated signaling to induce anti-fungal innate and adaptive immune responses.

SHP-2 in macrophages and neutrophils regulate anti-fungal innate immunity

To investigate the role of SHP-2 in macrophages and neutrophils during *C. albicans* infection, *Shp-2*$^{fl/fl}$ and M/N-*Shp-2*$^{-/-}$ mice were infected i.v. with *C. albicans*. M/N-*Shp-2*$^{-/-}$ mice had greater mortality at early stages of infection (Fig. 7a), and suffered more weight loss than *Shp-2*$^{fl/fl}$ mice (Fig. 7b). Consistently, M/N-*Shp-2*$^{-/-}$ mice produced less CXCL1 and CXCL2, but similar concentrations of TNF, IL-6 and IL-12, compared to *Shp-2*$^{fl/fl}$ mice (Fig. 7c). Interestingly, fungal-specific T_H17 and T_H1 responses were unimpaired in M/N-*Shp-2*$^{-/-}$ mice (Fig. 7d). Importantly, numbers of neutrophils and macrophages that infiltrated into the kidneys of M/N-*Shp-2*$^{-/-}$ mice were significantly reduced compared to the kidneys of *Shp-2*$^{fl/fl}$ mice 3 days after infection (Fig. 7e).

Furthermore, M/N-*Shp-2*$^{-/-}$ BMDMs exhibited impaired phagocytosis of zymosan particles (Supplementary Fig. 7). These results implicate that SHP-2-regulated C-type lectin signaling in macrophages and neutrophils contributes to early control of *C. albicans* infection.

DISCUSSIONS

This study identifies SHP-2 as an essential component responsible for the recruitment of Syk to dectin-1, dectin-2/3 and Mincle receptors, thereby mediating Syk activation and anti-fungal immune responses. Through genetic ablation of *Shp-2* in DCs and macrophages/neutrophils, we formally demonstrated that C-type lectin receptor-triggered signals in DC compartment play an indispensable role in the induction of anti-fungal T_H17 response. Collectively, our results unraveled fundamental mechanisms by which C-type lectin receptors orchestrate innate and adaptive immune responses in host defense against fungal pathogens.

In C-type lectin receptor signaling, tyrosine phosphorylation initiated by Src-like kinases and Syk is critical for activating NF-κB and MAPKs. We now show during fungal infection with *C. albicans*, SHP-2 is recruited to dectin-1 or dectin-2/3-associated FcRγ. Our data further suggest that tyrosine phosphorylation on the cytoplasmic tail of dectin-1 or the ITAM motif of FcRγ is necessary for SHP-2 recruitment. Once recruited to dectin-1 or dectcin-2/3, SHP-2 is tyrosine phosphorylated at the C-terminus, which then recruits Syk to dectin receptor complexes. The tyrosine residues Y_{511} and Y_{542} located at the SHP-2 C-terminus are essential for this because mutation of either Y_{511} or Y_{542} resulted in diminished SHP-2 tyrosine phosphorylation and abrogated Syk recruitment. Notably, the p-Y_{542}XXI of SHP-2 has been reported to mediate the recruitment of SH2-containing adaptor Grb2 in EGF-PDGF signaling[28]. Our study indicates that the p-Y_{542} XXI can also form an intact ITAM motif with the p-Y_{511}XXI, allowing SHP-2 to recruit Syk in C-type lectin receptor signaling, as well as antibody-triggered FcγR and BCR signaling. These observations are in line with the hypothesis that the region linking two SH2 domains of Syk is flexible enough to accommodate Syk binding to ITAM motifs with different lengths of spacer[23]. Nevertheless, more detailed structural and functional analyses are needed to understand precisely how this non-canonical ITAM motif of SHP-2 engages Syk, Grb2 and other SH2-containing molecules. While our data argue that the catalytic activity of SHP-2 is dispensable for the recruitment of Syk to dectin-1 and the activation of Syk, it is very likely that SHP-2 uses its phosphatase activity in other processes. Indeed, SHP-2 regulates IL-6 and IFN-γ production by dephosphorylating STAT3 and STAT1, respectively[30,33,36]. SHP-2 has recently been reported to dephosphorylate SIRPα, thereby regulating Zymosan-induced ROS production in macrophages[37]. Nevertheless, our studies and others[34] indicate that SHP-2 can regulate innate immune signaling in a phosphatase-independent manner.

Recent studies have revealed complex receptor interactions and adaptor usages in CLR family members, such as dectin-2, dectin-3 and Mincle. Dectin-2 and dectin-3 form heterodimers in the recognition of mannan[27], and dectin-3 and Mincle work in synergy in TDB-induced responses[38]. However, dectin-1 does not form heterodimers or work together with other receptor to sense β-glucan[7,25,39]. Unlike dectin-2/3 and Mincle, dectin-1 does not rely on FcRγ to trigger downstream signaling. The cytoplasmic tail of dectin-1 contains only

one half of the ITAM motif ($Y_{15}XXL$)[7,25], whereas Syk has to bind to at least one intact ITAM motif to be activated[23]. In this regard, it has been uncertain how dectin-1 activates Syk, however, the data presented here appears to resolve this paradox. Unlike Syk, we show here that SHP-2 can use either N-SH2 (for dectin-1) or both N-SH2 and C-SH2 (for FcRγ) for receptor/adaptor binding, demonstrating the structural and functional flexibility. In dectin-1 signaling, the SHP-2 N-SH2 domain engages the hemITAM of dectin-1. Conceivably, dimerization of dectin-1 would allow simultaneous recruitment of two SHP-2 molecules, which then act through ITAM-SH2-SH2 interaction to bring two Syk molecules to dectin-1. Subsequently, two Syk molecules in close proximity activate each other through inter-molecular autophosphorylation. On the other hand, both SH2 domains of SHP-2 associate with FcRγ, which contains an intact ITAM motif and usually forms dimers, allowing similar activation of Syk for dectin-2/3 signaling. It remains unclear why the ITAM motif of FcRγ is insufficient in recruiting Syk; however, one of the possibilities is that the long inter-region between the SH2 domains and the ITAM motif allows SHP-2 to overcome the space restriction that prevents Syk binding to dectin-2/3-FcRγ complex. Our finding that SHP-2 also mediates Syk recruitment in BCR and FcγR signaling implies that SHP-2-Syk interaction is widely used by immune regulatory signals. Considering multiple adaptors have to work together to trigger robust TCR signaling, the involvement of both SHP-2 and FcRγ in dectin-2/3, and likely Mincle signaling should not come as a surprise. This type of differential usage of adaptors among C-type lectin receptors is reminiscent of Toll-like receptors, which use either Myd88 for TLR5 or MyD88 and TRAM for TLR2[40].

Using DC-*Shp-2*-deficient mice, we demonstrated a critical role of DC-derived CLR signaling in anti-fungal T_H17 responses. DC-derived CLR signaling is required for the induction of IL-1-β, IL-6 and IL-23, which mediate T_H17 induction. Indeed, T_H17 responses were impaired in the spleen and kidney of DC-*Shp-2*$^{-/-}$ mice, leading to heightened fungal burden and more severe tissue damage. These mice also showed attenuated fungal-specific T_H1 responses *in vivo*, possibly due to reduced IL-12 production by DCs. Conversely, SHP-2-mediated signaling in macrophages and neutrophils primarily affects the production of chemokines CXCL1 and CXCL2, which play a critical role in recruiting neutrophils and monocytes. Notably, SHP-2-mediated signaling in macrophages and neutrophils had no significant impact on anti-fungal T_H17 and T_H1 responses *in vivo*. Accordingly, M/N-*Shp-2*$^{-/-}$ mice suffered greater mortality only at an early stage, underscoring the importance of infiltrating macrophages and neutrophils and phagocytosis to the anti-fungal innate immune response. These results clearly indicate that CLR signaling in different cellular compartments have distinct roles in the induction of anti-fungal innate and adaptive immune responses.

SHP-2 plays a variety of roles in development, hematopoiesis and tumorigenesis through the regulation of signals induced by growth factors, hormones and cytokines[28,30]. Here, our data extend the role of SHP-2 to host defense against fungal pathogen. Gain of function mutations in *Shp-2* gene are associated with Noonan syndrome, JMML and Leopard syndrome[41,42], therefore, identification of polymorphisms or loss-of-function mutations in *Shp-2* linking to the susceptibility to human candidiasis will be critical for establishing a role of SHP-2, along with dectin-1 and CARD9, to human anti-fungal immunity[43,44]. In addition,

CLRs are also capable of sensing the cell wall components from mycobacteria[9,45], Leishmania[46] as well as endogenous ligands SAP130[47] and mucin[48]. Therefore our results suggest there may be a more general role for SHP-2 in host defense and maintenance of immune homeostasis.

In summary, we identified a novel role for SHP-2 in the assembly of C-type lectin receptor complexes for Syk activation and anti-fungal immune responses. This study also formally demonstrates a pivotal role for DC-derived C-type lectin receptors in the initiation of anti-fungal T_H17 responses, highlighting the importance of concerted actions from different CLRs on various innate immune cells to host defense against fungal pathogens.

METHODS

Mice

Shp-2 floxed mice, FcRγ-deficient (*Fcer1g*$^{-/-}$) mice (obtained from J.V. Ravetch, The Rockefeller University), dectin-1-deficient (*Clec7a*$^{-/-}$) mice (obtained from Y. Iwakura, The University of Tokyo) and Mincle-deficient (*Clec4e*$^{-/-}$) mice (obtained from S. Yamasaki, Kyushu University, Japan) were constructed as previously described[47,49,50]. *Cd11c*-cre, *Vav*-cre and *LysM*-cre mice were purchased from Jackson Laboratories (Bar Harbor, ME). These mice were bred and maintained in a pathogen-free animal facility at Institut Pasteur of Shanghai. All the procedures were conducted in compliance with a protocol approved by the Institutional Animal Care and Use Committee at Institut Pasteur of Shanghai.

Plasmids and reagents

The cDNA for SHP-2 and its mutants SHP-2ΔN-SH2, SHP-2ΔC-SH2 were amplified from C57BL/6 bone marrow cells by various sets of primers (Supplementary Table 1) and cloned into pCDH vector by enzymes *Nhe* I and *EcoR* I. Dectin-1 and its mutants dectin-1 Y15F were amplified from C57BL/6 bone marrow cells and cloned into pcDNA3.1 vector through *EcoR* I and *Not* I. Syk and its mutants SykΔN-SH2, SykΔC-SH2 were cloned from C57BL/6 bone marrow cells by various sets of primers (Supplementary Table 1) and cloned into pcDNA3.1 vector through *EcoR* I and *Xba* I. The cDNA for dectin-3 was amplified from C57BL/6 bone marrow cells and cloned into pcDNA3.1 vector through *BamH* I and *Xho* I. The cDNA for FcRγ was amplified from C57BL/6 bone marrow cells and cloned into pcDNA3.1 vector through *EcoR* I and *Not* I. SHP-2 Y542F and Y511F mutants were generated by primers listed in Supplementary Table 1 by overlapping approach.

Zymd (Zymosan depleted (cat. tlrl-dzn), *Saccharomyces cerevisiae* cell wall preparation treated with hot alkali to remove all its TLR-stimulating properties), Curdlan, Zymosan A (cat. tlrl-zyn) were purchased from Invivogen; Zymosan A-Alexa Fluor®594 (cat: Z23374) was from Invitrogen; mannan and TDB were from Sigma and Avanti Polar Lipids, respectively. GM-CSF and IL-4 were from R&D systems. Antibody information is listed in Supplementary Table 2.

C. albicans culture and heat inactivation

Single colony of *C. albicans* SC5314 from yeast peptone dextrose agar plate was inoculated into the yeast peptone dextrose medium and cultured overnight at 25°C (yeast forms of *C. albicans*). To prepare hyphae, yeast cells were cultured in yeast peptone dextrose medium plus 10% FCS for 3 h at 37°C. Yeast or hyphae cells were washed with phosphate-buffered saline three times, and resuspended in PBS buffer followed by incubation for 1 h at 65°C to kill fungi.

Generation of bone marrow-derived DCs and macrophages

Bone marrow cells were isolated by flushing femurs and tibia of 6–8 week C57BL/6 mice with RPMI 1640 medium (Invitrogen). Red blood cells were lysed using ACK lysis buffer (0.15M NH_4Cl, 1mM $KHCIO_3$, 0.1mM Na_2EDTA, pH7.3). IL-4 (10ng/ml, R&D systems) and GM-CSF (20ng/ml, R&D systems) were used to generate BMDCs in 1640 medium containing 10% FBS (HyClone), 2mM L-glutamine and 200μM β-mercaptoethanol. Media containing GM-CSF and IL-4 were replaced every two days. On day 9, nonadherent cells were collected by centrifugation and then resuspended in fresh medium containing GM-CSF for use. BMDCs differentiated by GM-CSF (10ng/ml, R&D systems) without IL-4 for 9 days were used for mannan stimulation (Fujikado et al., 2008). To generate BMDMs, bone marrow cells were cultured in RPMI 1640 medium supplemented with 30% L929-conditioned medium (containing M-CSF), as well as 10% FBS. At day 4, non-adherent cells were removed and fresh RPMI and L929-conditoned medium was added. BMDMs were used on day 7–10.

Immunoprecipitation and Western blotting

Cells were lysed in lysis buffer (50mM Tris, pH7.4, 150mM NaCl, 1% Triton X-100, 1mM EDTA, pH8.0) supplemented with protease inhibitor complete mini (Roche) and 1mM PMSF, 1mM Na_3VO_4, 1mM NaF, and cell debris was cleared by centrifugation at 13Krpm for 15 min. The cell lysates were immunoprecipitated by 1–2μg antibodies overnight followed by Protein A sepharose™ 6MB beads (GE Healthcare Life Sciences, 17-0469-01) incubation for 1 h at 4°C. The immunoprecipitates were washed three times with wash buffer (50mM Tris, pH8.0, 300mM NaCl, 1% Triton X-100, 1mM EDTA and 0.1%SDS). Proteins were eluted with 2 × SDS loading buffer. After boiling for 10 min the samples were fractionated on 10% SDS-PAGE and transferred to PVDF member and probed with indicated antibodies.

C. albicans infection

Live *C. albicans* SC-5314 (2×10^5 or 1×10^6 yeast cells in 0.1ml of PBS buffer) were intravenously injected into 6–8 weeks old littermates of distinct genotypes. Infected mice were monitored daily for their weight loss and survival. Fungal burden was measured 5 days after infection. Following harvesting kidneys, livers, and spleens, tissue homogenates were serially diluted and plated on yeast extract peptone dextrose agar. Fungal colony formation units were counted after 24 hours. For histological analysis, kidneys were fixed by 10% formalin and paraffin-embedded sections were stained by periodic acid-Schiff or hematoxylin and eosin. For immune staining, kidneys were snap-frozen in OCT and frozen

sections were stained by anti-Gr-1 (#550291, BD Pharmingen) and anti-F4/80 (#14-4801, eBioscience), respectively.

ELISA

BMDCs (differentiated by GM-CSF or by GM-CSF plus IL-4) were stimulated by mannan, Zymd, Zymosan A, Curdlan and heat-inactivated fungi, respectively for 24 h. BMDMs were either primed by GM-CSF (10ng/ml) or IL-4 (10ng/ml) overnight, followed by stimulation by mannan or Zymd, Zymosan A, Curdlan and HKCA, respectively, for 24 h. Supernatant were harvested and the secreted amounts of TNF, IL-1β, IL-6, IL12p40, IL12p70, IL12p19, CXCL-1, CXCL-2 were measured by ELISA kits (eBioscience, R&D systerms) according to manufacturers' recommendations. Following i.v. infection by 2×10^5 or 1×10^6 live *C. albicans* SC5314, mice sera were collected in 24 h and the production of cytokines and chemokines was measured by ELISA.

Splenic T cell response

Five days after *C. albicans* infection, mice were sacrificed and total spleens were harvested and homogenized into single cells. Splenic cells were plated on 96-well plate (1×10^6 cells/well) cultured with RPMI 1640 containing 10% FBS (HyClone) and restimulated by 1×10^6 cells heat-killed *C. albicans* for 48 h. Supernatants were collected for the measurement of IL-17 and IFN-γ concentrations by ELISA.

Tissue RNA preparation and Real-time PCR

RNAs were extracted from Kidney using TRIZOL (Invitrogen) according to the manufacture's instruction. cDNA was reversely transcribed from 0.5μg total RNA by PrimeScript™ RT-PCR Kit (Takara). Real-time PCR were carried out with PrimeScript® RT reagent Kit (Takara) on ABI 7900HT Fast Real-time PCR System. Relative expression of target genes was quantitatively normalized against the expression of *GAPDH* using ΔΔct method. All Real-time PCR primers used in this study are described on Supplementary Table 3.

Lentivirus preparation and infection

Lenti-viral vector pCDH expressing wild-type or mutants of SHP-2 were transiently transfected into HEK293T cells along with packaging plasmids (Δ8.91/VSV-G), and viruses-containing media were harvested in 48 h. Lentiviruses were added onto bone marrow cells cultured with RPMI 1640 medium supplemented with 30% L929-conditioned medium, as well as 10% FBS on day 3. After incubation for 12 h, virus-containing medium was removed and substituted with fresh medium in the presence of 2μg/ml puromycin. Stable pools of infected BMDMs were generated in 7 days and re-plated for stimulation by Zymd overnight.

Cell fractionation

BMDCs stimulated with Zymd for various times were lysed in 1 ml Buffer A (50mM Tris-HCl, pH7.4, 150mM NaCl, 1mM EDTA, supplemented with protease inhibitor (complete mini, Roche) and 1mM PMSF, 1mM Na_3VO_4, 1mM NaF) by sonication. Following

centrifugation for 5 min at 1Krpm, nuclei-containing pellets were discarded, and supernatants were subject to ultra-centrifugation at 60Krpm for 1 h. Following ultra-centrifugation, supernatants were collected as cytosolic fraction; pellets were washed twice with buffer A and then lysed in 200μl buffer B (50mM Tris, pH7.4, 150mM NaCl, 1% Triton X-100, and 1mM EDTA) supplemented with protease inhibitor and phosphatase inhibitors by vortexing. After removing insoluble materials by centrifugation, soluble proteins were collected as cell plasma membrane fraction.

Statistic analysis

Log-rank (Mantel-Cox) Test and Student's T test were used for statistical analysis and $p<0.05$ is considered significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKOWLEDGEMENTS

We are grateful to Bangguo Qian from the Pathology Core Facility of Institut Pasteur of Shanghai for the technical assistance in histology, and Yamin Xu and Dr. Jianshe Yan from Shanghai Institute of Immunology, Shanghai Jiaotong University School of Medicine for their technical assistance in confocal microscopy. This study is support by National Science and Technology Major Projects 2014CB541902 (to H.X.) and 81270627 (to H.H.Z); National Natural Science Foundation of China grants 31070779 and 31170862 (to H.X.), as well as 31270917 (to M.D.) and 31100623 (to A.Z.); NIHR01HL096125 (to G-S.F.); NIHP01 HL103453 (to X.X.L.) and NIHR01EY18612 (to E.P.). H.X. is supported by Chinese Academy of Sciences "100-talent" program and National program for returned oversea talents and H.H. Zhu is supported by Science and Technology Commission of Shanghai Municipality (Pujiang program 12PJ1406100).

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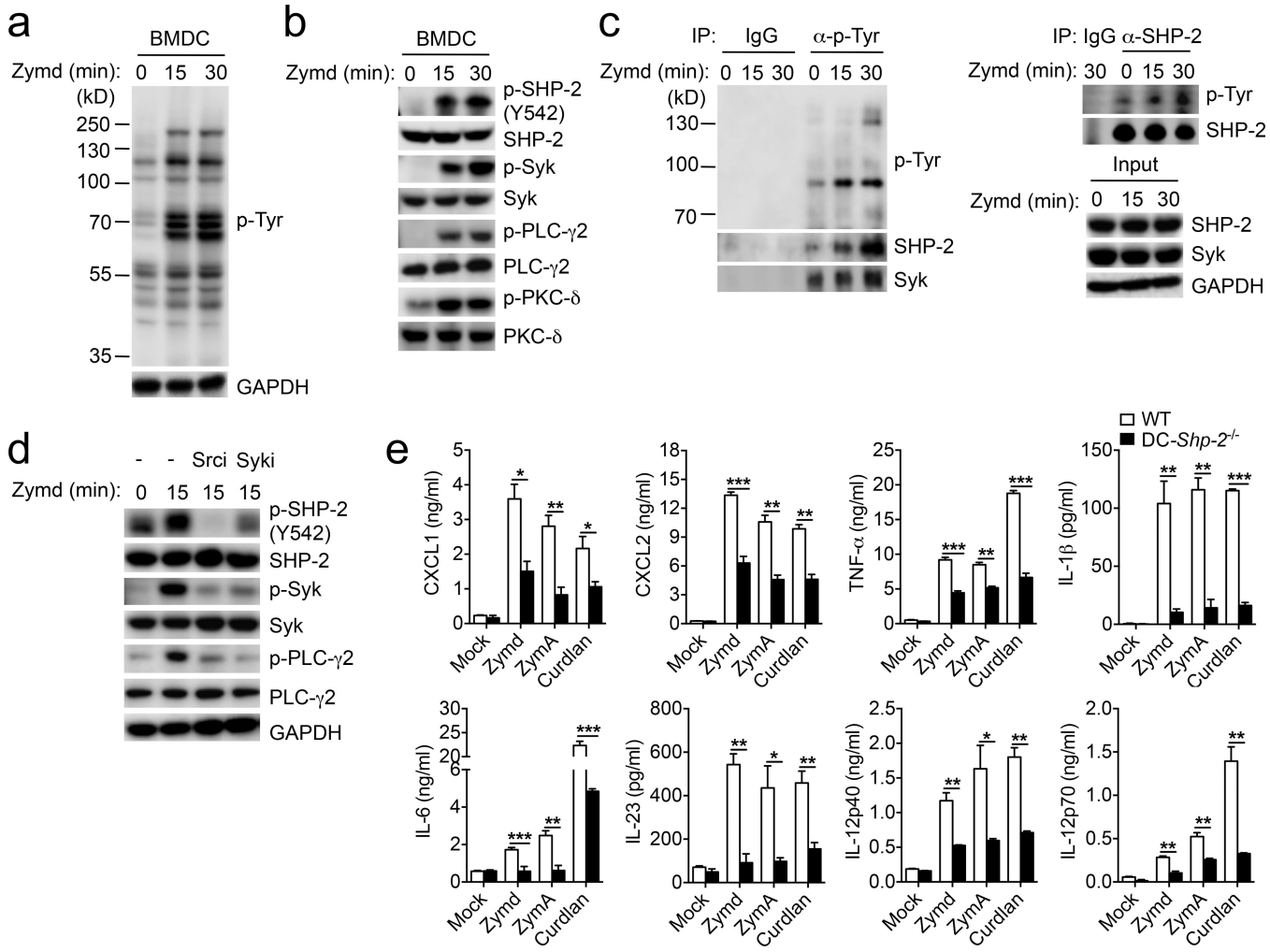

Figure 1.

SHP-2 is tyrosine-phosphorylated and regulates pro-inflammatory gene expression upon dectin-1 activation. (**a,b**) Wild-type BMDCs differentiated by GM-CSF (20ng/ml) and IL-4 (10ng/ml) were either untreated or treated by Zymd (100μg/ml) for various times as indicated. Cell lysates were immunoblotted by respective antibodies. (**c**) Wild-type BMDMs primed with IL-4 (10ng/ml) overnight were either untreated or treated by Zymd (100μg/ml). Cell lysates were immunoprecipitated by control IgG, anti-SHP-2 or anti-p-Tyr, followed by immunoblotting with anti-p-Tyr or anti-SHP-2, respectively. (**d**) Wild-type BMDCs were pretreated by vesicle or inhibitors PP2 (3μM) and Piceatannol (15μM) for 1 h, followed by Zymd treatment for 15 min. Cell lysates were probed by indicated antibodies. (**e**) BMDCs derived from *Shp-2*$^{fl/fl}$ and DC-*Shp-2*$^{-/-}$ mice were stimulated by dectin-1 ligands Zymd (100μg/ml), ZymA (100μg/ml) or Curdlan (100μg/ml) for 24 h. Supernatants were collected and amounts of cytokines and chemokines were measured by ELISA. Data are presented as means ± SEM from three samples for each group in one representative experiment, and similar results were obtained from three independent experiments. * p<0.05, ** p<0.01, *** p<0.001.

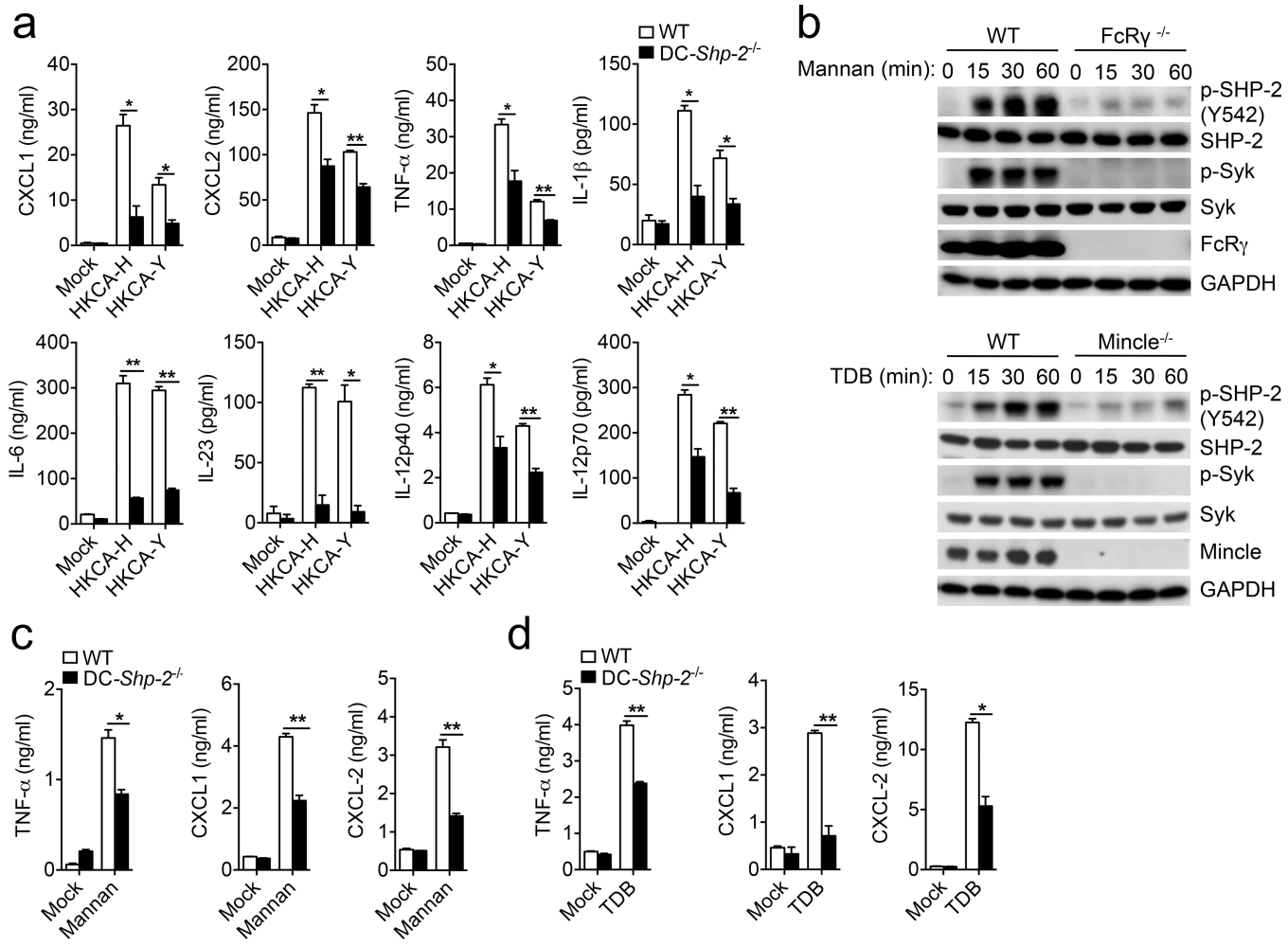

Figure 2.

SHP-2 is required for CLR- and *C. albicans*-induced gene expression. (**a**) BMDCs derived from *Shp-2*$^{fl/fl}$ and DC-*Shp-2*$^{-/-}$ mice were stimulated by heat-killed yeast (MOI: 2) or hyphae of *C. albicans* (MOI: 1) for 24 h. (**b**) BMDCs derived from WT, FcRγ-deficient or Mincle-deficient mice were stimulated by mannan (100μg/ml) or TDB (50μg/well) for indicated times. Whole cell lysates were resolved by 10% SDS-PAGE and then probed by respective antibodies. (**c,d**) Wild-type and DC-*Shp-2*$^{-/-}$ BMDCs were stimulated by mannan (100μg/ml) or TDB (50μg/well) for 24 h. Supernatants were collected and amounts of cytokines and chemokines were measured by ELISA. Data are presented as means ± SEM from three samples for each group in one representative experiment of three. * p<0.01, ** p<0.001.

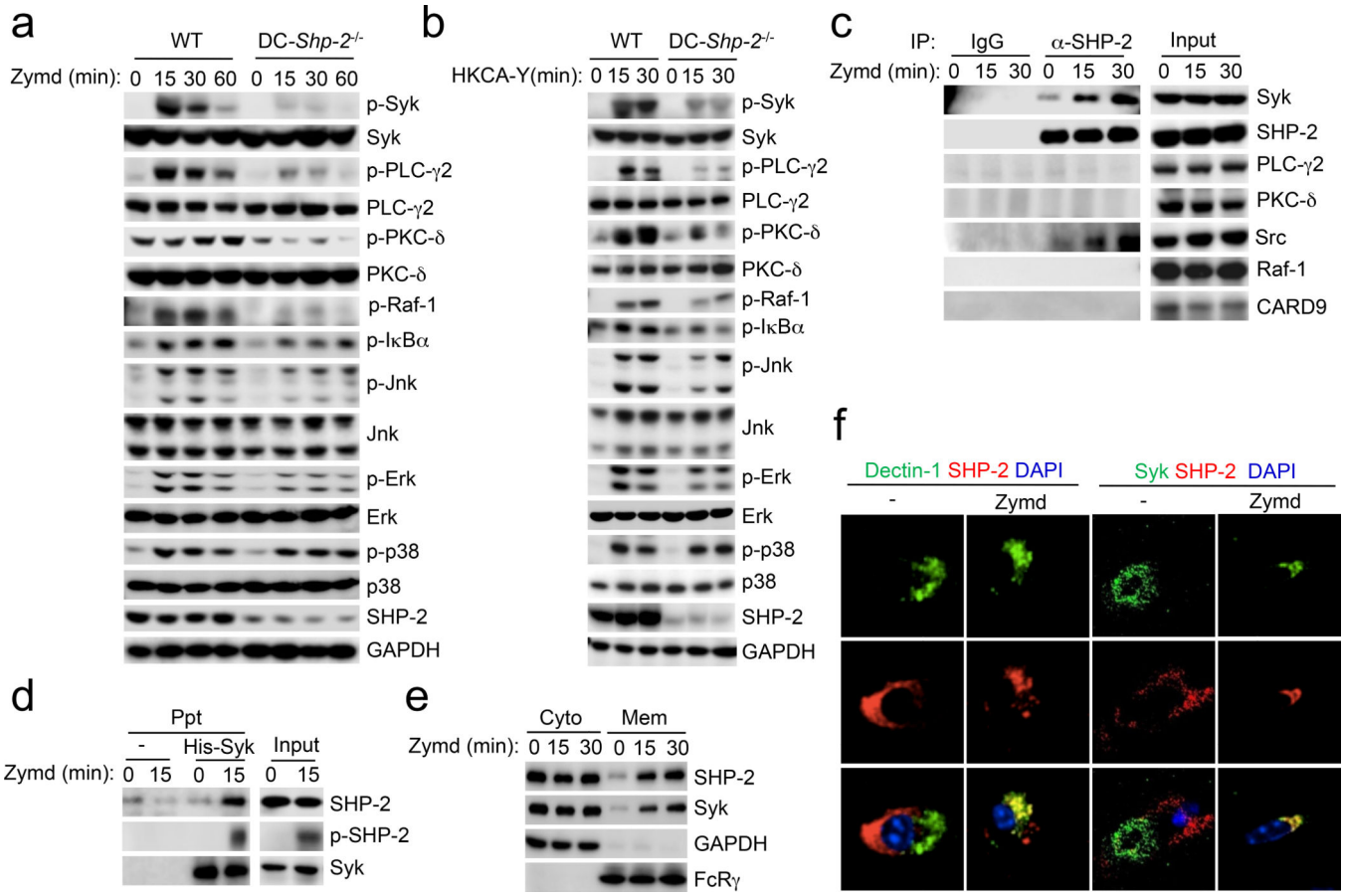

Figure 3.

SHP-2 mediates Syk activation in dectin-1 and *C. albicans*-induced signaling. (**a,b**) BMDCs were either untreated or treated by Zymd (100μg/ml) (**a**) or heat-killed *C. albicans* yeast (MOI: 2) (**b**), and cell lysates were immunoblotted by indicated antibodies. (**c**) BMDCs were either untreated or treated by Zymd (100μg/ml) for various times, and cell lysates were immunoprecipitated by control IgG or anti-SHP-2. (**d**) BMDCs were either untreated or treated by Zymd (100μg/ml) for 15 min before lysis. Whole cell lysates were incubated with nickel-beads or nickel-beads conjugated His-tagged Syk protein (1μg) for 4 h. Proteins pulled-down were probed by indicated antibodies. (**e**) BMDCs treated by Zymd for various times were subject to biochemical fractionation and cytosolic and cell membrane proteins were probed by anti-SHP-2 and anti-Syk, respectively. (**f**) Untreated or Zymd-treated BMDCs were fixed by paraformaldehyde and stained by indicated antibodies and DAPI. Representative data from three independent experiments are shown.

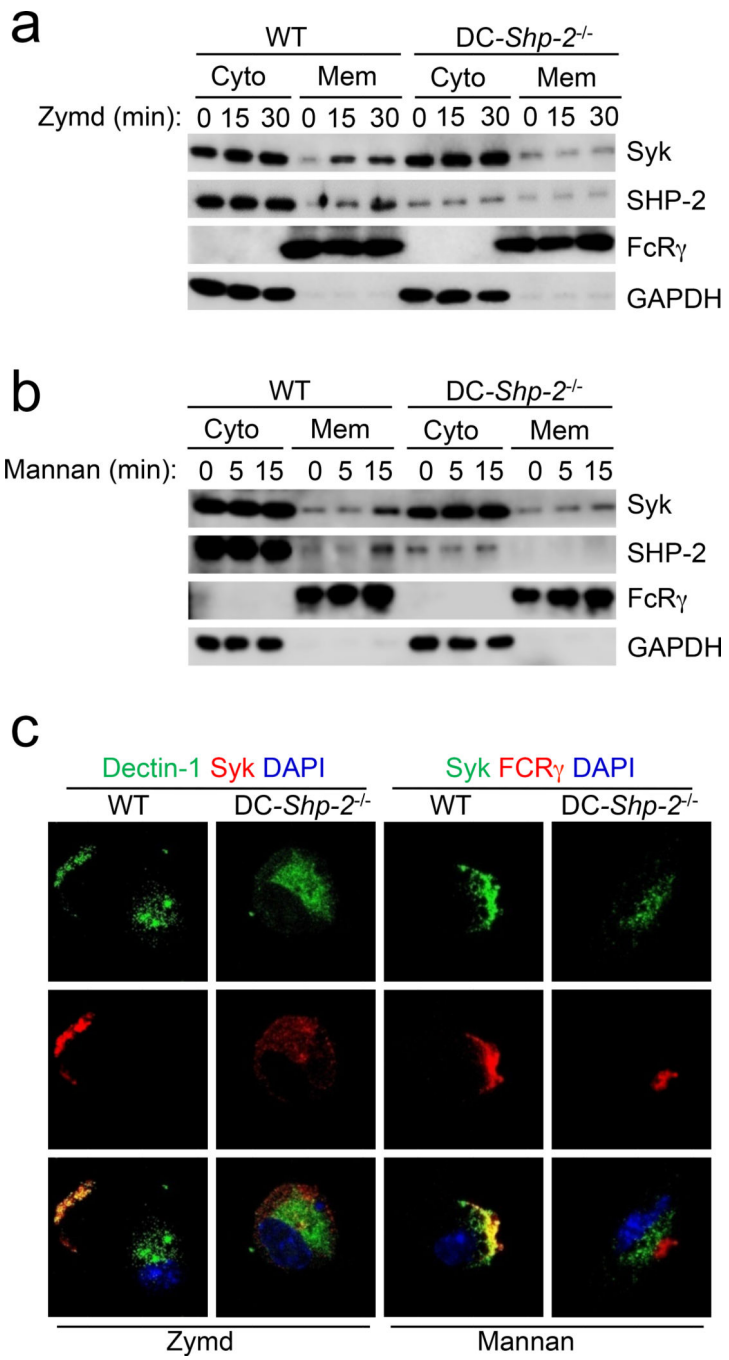

Figure 4.

SHP-2 mediates Syk membrane translocation and colocalization with dectin-1 and FcRγ. (**a**) BMDCs derived from *Shp-2*$^{fl/fl}$ and DC-*Shp-2*$^{-/-}$ mice were stimulated by Zymd or Mannan for various times. Cytosolic and cell membrane fractions were resolved by SDS-PAGE and probed with anti-Syk. (**b,c**) WT and DC-*Shp-2*$^{-/-}$ BMDCs were stimulated with Zymd (**b**) or mannan (**c**) for 10 min, followed by immune fluorescence staining with indicated antibodies. Representative data from two independent experiments are shown.

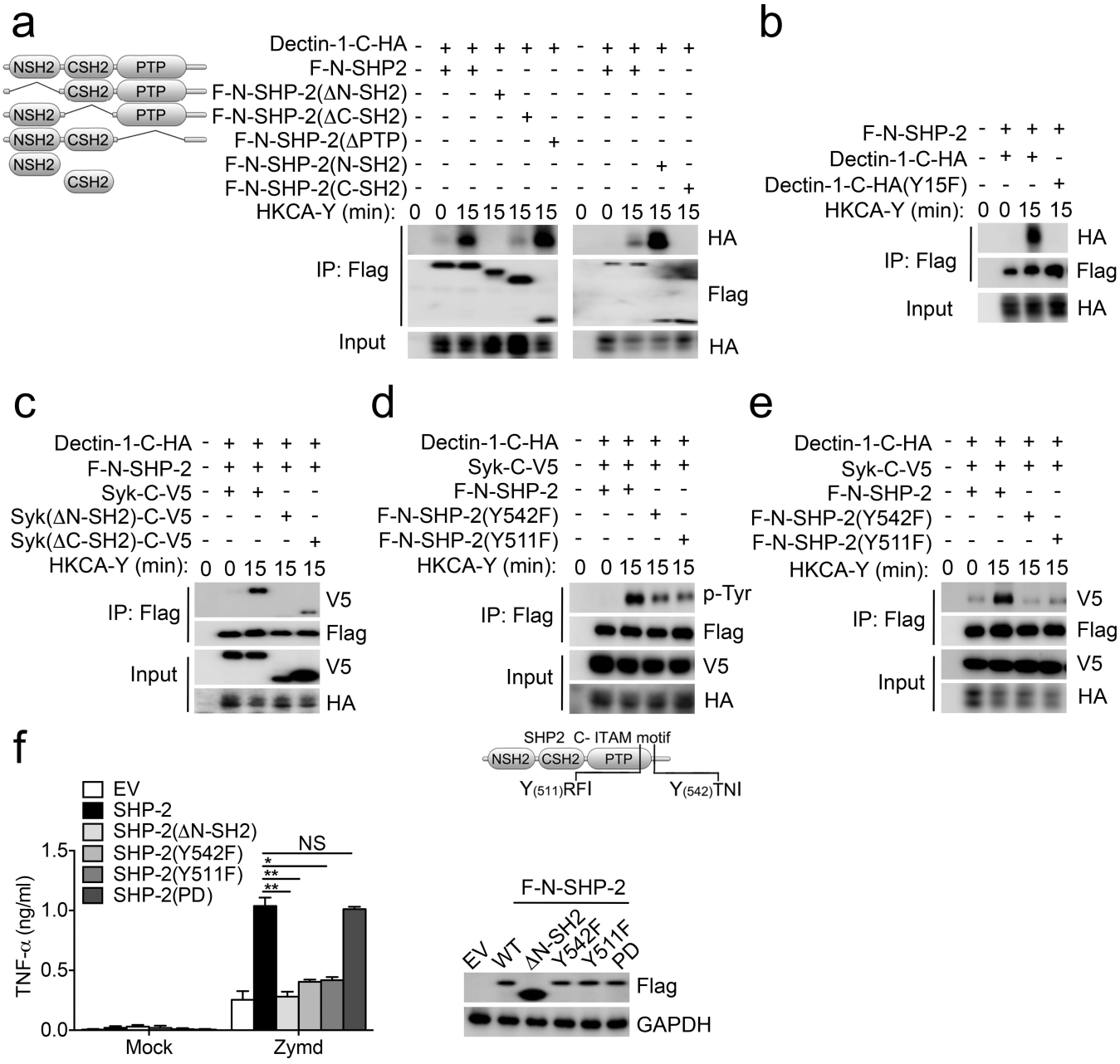

Figure 5.

SHP-2 recruits Syk to dectin-1 upon *C. albicans* infection. (**a,b**) HEK293T were transiently transfected by plasmids expressing FLAG-tagged wild-type or various deletion mutants of SHP-2, along with plasmids expressing HA-dectin-1 or HA-dectin-1 Y15F. In 48 h, cells were left unstimulated or stimulated by heat-killed *C. albicans* yeast (MOI: 2) for 15 min. Cell lysates were immunoprecipitated by anti-FLAG and probed by indicated antibodies. (**c,d,e**) HEK 293T cells were transiently transfected by plasmids expressing V5-tagged wild-type and N-SH2 or C-SH2 deletion mutants of Syk along with plasmids expressing HA-tagged dectin-1 and Flag-tagged SHP-2 (**c**); or V5-tagged Syk and HA-tagged dectin-1, along with Flag-tagged wild-type and mutants of SHP-2 (**d,e**). 48 h after co-transfection,

cells were stimulated with heat-killed *C. albicans* yeast (MOI: 2) for 15 min. Cell lysates were immunoprecipitated by anti-FLAG and probed by anti-HA or anti-p-Tyr. The representative results from three independent experiments were shown above. (**f**) BMDMs from M/N-*Shp-2*$^{-/-}$ mice were transducted by lentiviral vector pCDH expressing wild-type or mutants of SHP-2. Stable pools of lentiviral-transducted cells were primed by IL-4 (10ng/ml) and stimulated by Zymd (100μg/ml) for 24 h, TNF induction was measured by ELISA. Data are presented as mean ± SEM of three samples for each group, and the representative data from two independent experiments are shown. * $p<0.01$, ** $p<0.001$.

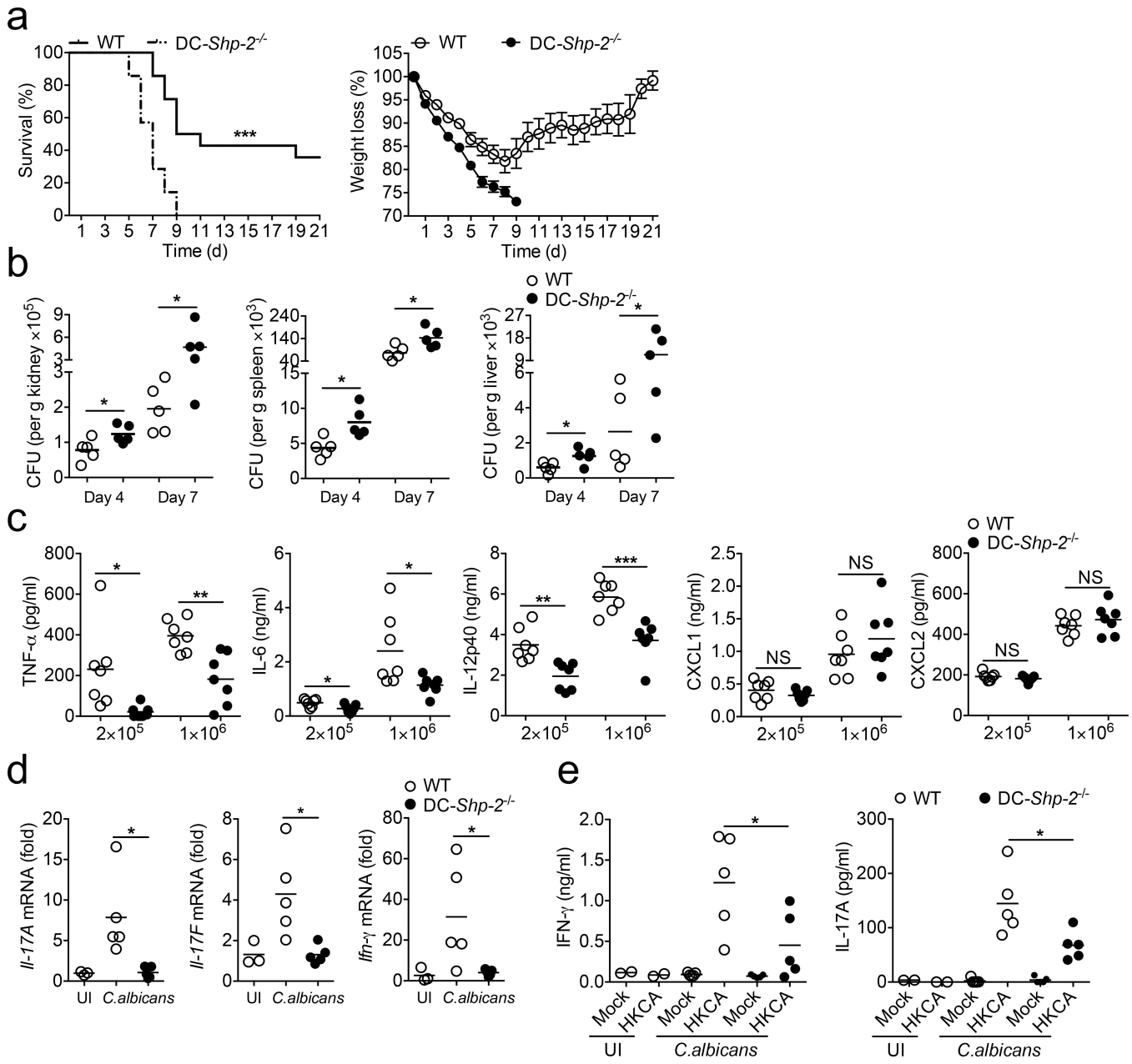

Figure 6.

SHP-2-mediated CLR signaling in DCs is indispensable for anti-fungal T_H17 responses. (**a**) 6–8 week-old littermates of *Shp-2*$^{fl/fl}$ and DC-*Shp-2*$^{-/-}$ mice were infected by *C. albicans* SC-5314 (2×10^5 fungal cells/mouse) via i.v. injection. Weight loss and survival were monitored daily. Data was pooled from 14 pairs of sex- and age-matched littermates through three independent experiments. Statistical analysis was performed by log-rank test ($p<0.0001$). (**b**) Kidneys, livers and spleens from infected mice were homogenized, and colonies of *C. albicans* SC-5314 were counted by serial dilution. (**c**) Mice were infected by *C. albicans* SC-5314 (2×10^5 or 1×10^6 fungal cells/mouse), and sera were collected in 24 h for ELISA. (**d**) Kidneys from *C. albicans* SC-5314 (2×10^5 fungal cells/mouse) infected mice

for 5 days were homogenized, followed by RNA isolation. IL-17A, IL-17F and IFN-γ mRNAs were assessed by quantitative real-time PCR. (**e**) Total spleens were isolated from mice uninfected or infected by *C. albicans* SC-5314 (2×10^5 fungal cells/mouse) for 5 days. Splenic cells were stimulated by heat-killed *C. albicans* (MOI: 1) for 2 days. IL-17A and IFN-γ from supernatants were measured by ELISA. UI: Uninfected. Data are presented as mean ± SEM from 5 mice per group, and representative data from three independent experiments are shown. Note: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

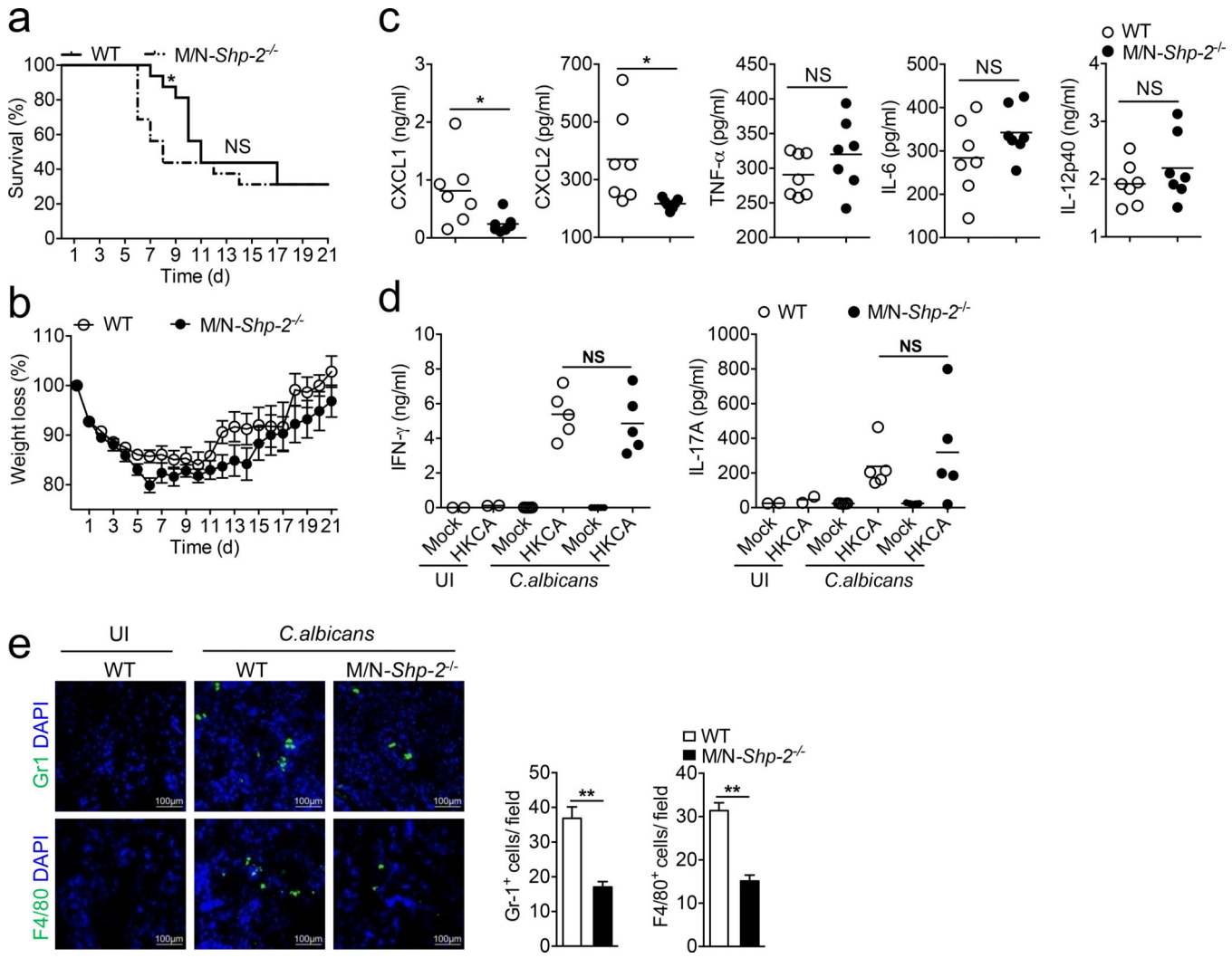

Figure 7.

SHP-2-mediated CLR signaling in macrophages/neutrophils is critical for anti-fungal innate immune responses. (**a,b**) 6–8 week-old littermates of *Shp-2*$^{fl/fl}$ and M/N-*Shp-2*$^{-/-}$ mice were infected by *C. albicans* SC-5314 (2×10^5 fungal cells/mouse) via i.v.. Weight loss and survival were monitored daily. Data was pooled from 16 pairs of sex- and age-matched littermates through three independent experiments. Statistical analysis was performed by log-rank test (day1–9, $p<0.05$; day10–21, $p>0.05$). (**c**) 24 h after *C. albicans* SC-5314 infection, sera were collected for ELISA. Data are presented as mean ± SEM from 7 mice per group, and representative data from two independent experiments are shown. (**d**) Spleens were isolated from mice uninfected or infected by *C. albicans* SC-5314 (2×10^5 fungal cells/mouse) for 5 days. Splenic cells were stimulated by heat-killed *C. albicans* (MOI: 1) for 2 days. Supernatants were collected for ELISA. Data are presented as mean ± SEM from 5 mice per group, and representative data from two independent experiments are shown. (**e**) Kidneys from *Shp-2*$^{fl/fl}$ and M/N-*Shp-2*$^{-/-}$ mice 3 days after *C. albicans* SC-5314 infection were embedded in OCT, and frozen-sections were stained by anti-Gr-1, anti-F4/80 and DAPI. Representative images were shown and positive cells for each genotype were

quantified over 20 fields from three independent samples. UI: Uninfected. Note: * $p<0.05$, ** $p<0.001$.