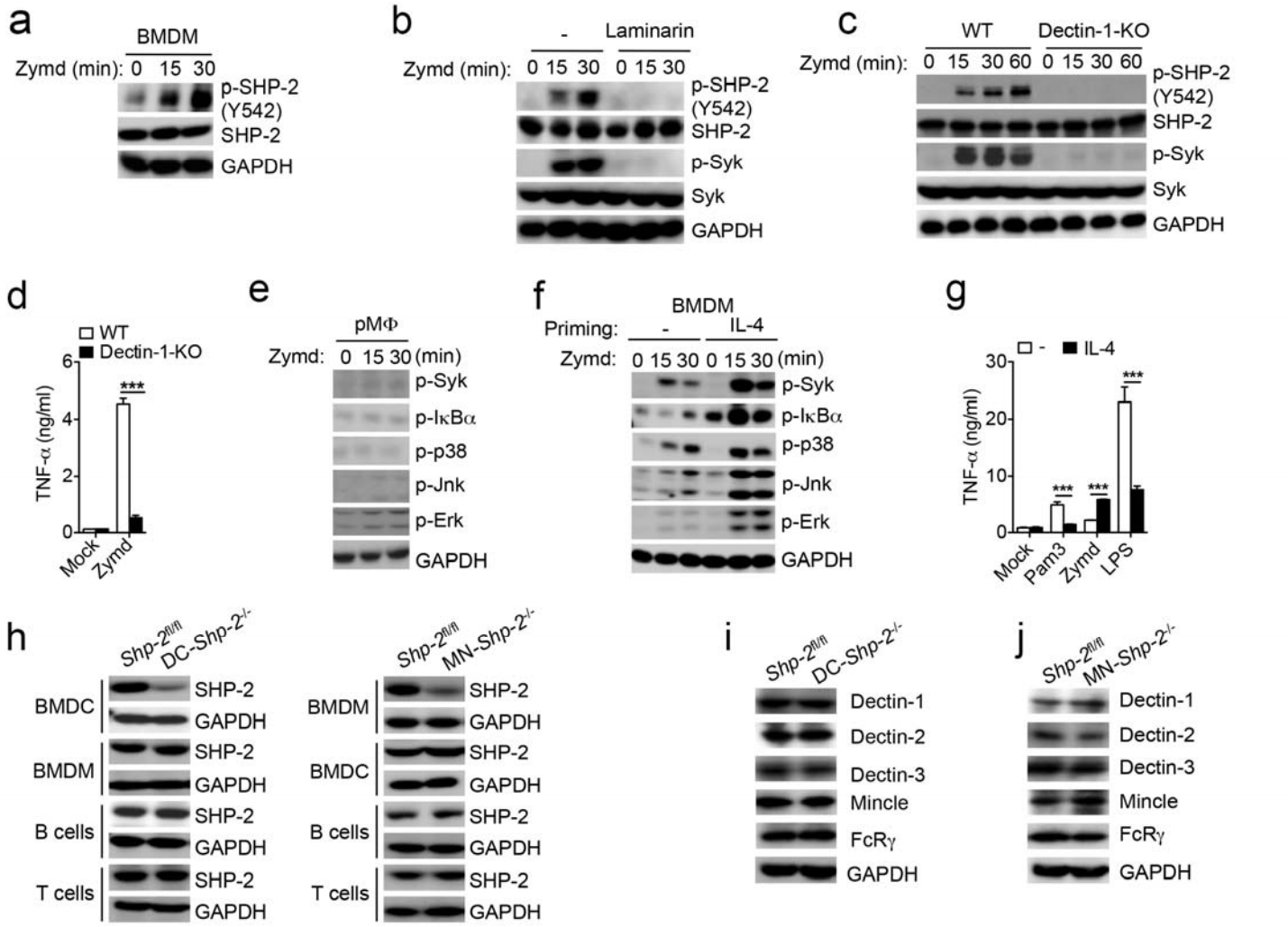
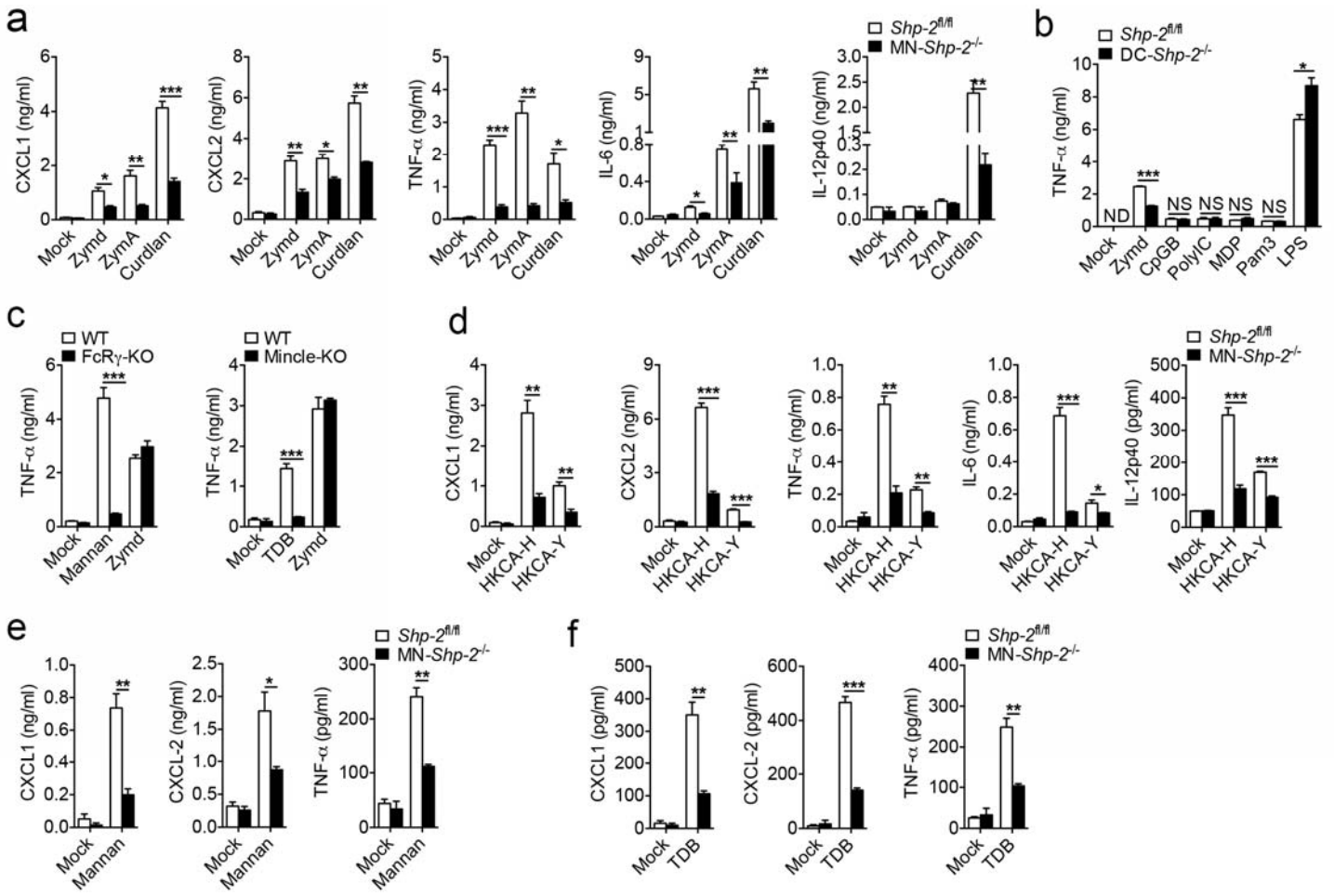


Supplementary Figure 1

SHP-2 is tyrosine-phosphorylated and regulates pro-inflammatory gene expression after stimulation with dectin-1 ligands.

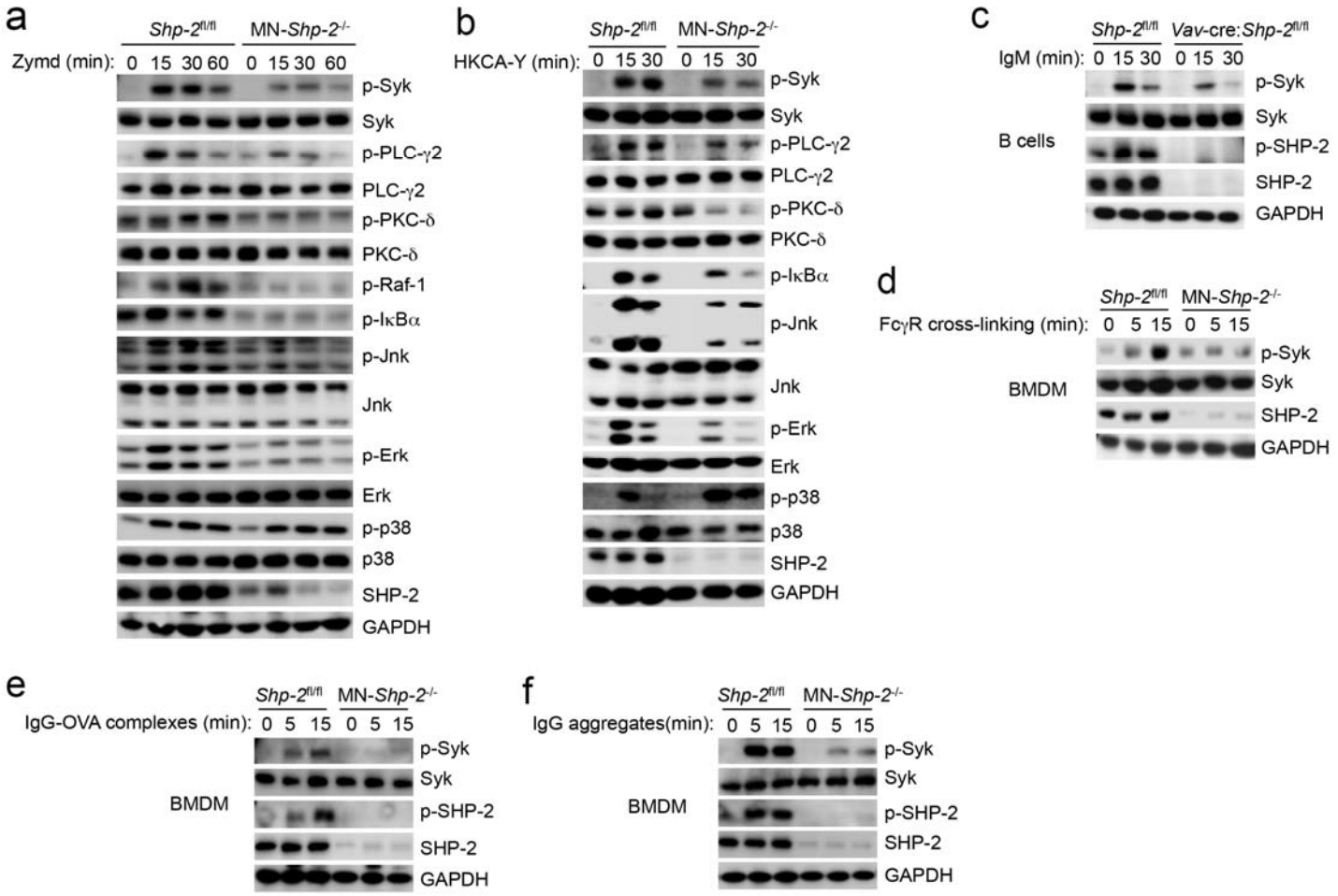
(**a**) Wild-type BMDMs primed with IL-4 (10ng/ml) overnight were either untreated or treated by Zymd (100μg/ml) for various times and Immunoblotting was conducted by respective antibodies as indicated. (**b,c**) BMDCs were untreated or pretreated by laminarin for 30 min, followed by 100μg/ml Zymd treatment (**b**); WT and dectin-1$^{-/-}$ BMDCs differentiated by GM-CSF (20ng/ml) and IL-4 (10ng/ml) were stimulated by Zymd (100μg/ml) (**c**). Immunoblot analysis was conducted with anti-p-SHP2 and anti-p-SYK. (**d**) BMDCs from WT and dectin-1 deficient mice (dectin-1-KO) were stimulated by Zymd (100μg/ml) for 24 h, supernatants were collected for ELISA. (**e**) Peritoneal macrophages were isolated from wild-type mice intraperitoneally injected with 4% Thioglycolate broth in PBS for 4 days, and stimulated by Zmyd (100μg/ml) for various times. (**f**) BMDMs with or without IL-4 (10ng/ml) priming overnight were either untreated or treated by Zymd (100μg/ml). (**g**) BMDCs differentiated by GM-CSF (20ng/ml) w/ or w/o IL-4 (10ng/ml) were stimulated with Pam3 (100ng/ml), Zymd (100μg/ml), or LPS (100ng/ml) for 24 hours and secreted TNF was measured by ELISA. (**h**) Cell lysates from BMDMs, BMDCs, spleen B cells and T cells generated from *Shp-2*$^{fl/fl}$, DC-*Shp-2*$^{-/-}$ and MN-*Shp-2*$^{-/-}$ mice were probed by anti-SHP2 and anti-GAPDH. (**i**) Cell lysates from BMDCs generated from *Shp-2*$^{fl/fl}$ and DC-*Shp-2*$^{-/-}$ mice were probed by indicated antibodies. (**j**) Cell lysates from BMDMs generated from *Shp-2*$^{fl/fl}$ and MN-*Shp-2*$^{-/-}$ mice were probed by indicated antibodies. Data are presented as mean ± SEM from three samples of one representative experiment of three. Note: * p<0.05, ** p<0.01, *** p<0.001.



Supplementary Figure 2

SHP-2 plays a critical role in dectin-1- and *C. albicans*–induced gene expression.

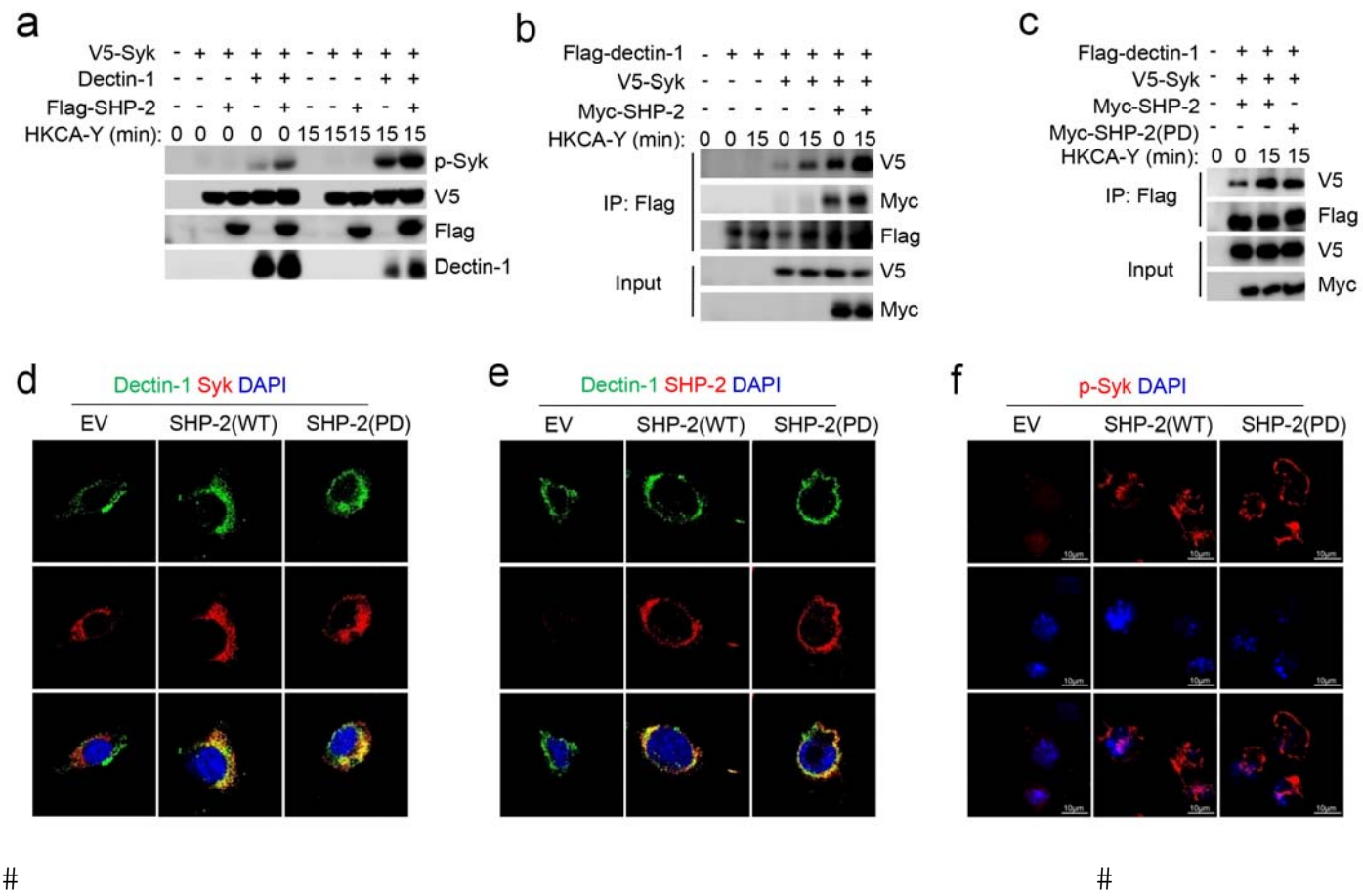
(**a**) BMDMs primed by IL-4 (10ng/ml) were stimulated by dectin-1 ligands Zymd (100μg/ml), ZymA (100μg/ml) or Curdlan (100μg/ml) for 24 h. Supernatants were collected for ELISA. (**b**) BMDCs were stimulated by Zymd (100μg/ml), MDP (10μg/ml), Pam3 (100ng/ml), CpGB (100nM), polyI:C (100μg/ml), or LPS (100ng/ml) for 24 h, and supernatants were collected for ELISA. (**c**) WT, FcRγ-KO and Mincle-KO BMDCs were stimulated with mannan (100μg/ml), TDB (50μg/well) or zymd (100μg/ml) for 24 h and secreted TNF was measured by ELISA. (**d**) BMDMs primed by IL-4 (10ng/ml) were stimulated by heat-killed yeast (MOI: 2) or hyphae of *C. albicans* (MOI: 1) for 24 h. Cytokines and chemokines were measured by ELISA. (**e,f**) BMDMs primed by GM-CSF (10ng/ml) were stimulated by dectin-2 ligand mannan (**e**) or Mincle ligand TDB (**f**) for 24 h. Supernatants were collected for ELISA. Data are presented as mean ± SEM from 3 samples for each group, and one representative experiment of three is presented. Note: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



Supplementary Figure 3

SHP-2 mediates Syk activation in a variety of signaling pathways.

(**a,b**) BMDMs primed by IL-4 (10ng/ml) were either untreated or treated by Zymd (100μg/ml) (**a**) or heat-killed *C. albicans* yeast (MOI, 2) (**b**) and cell lysates were probed by indicated antibodies. (**c**) B cells were isolated from *Shp-2*$^{fl/fl}$ or *Vav*-cre:*Shp-2*$^{fl/fl}$ spleens. Purified B cells were seeded into 12-well plate and stimulated with IgM (10μg/ml). (**d**) BMDMs from *Shp-2*$^{fl/fl}$ or MN-*Shp-2*$^{-/-}$ mice were incubated with anti-CD16/32 antibody (2.4G2, BD Biosciences,10μg/ml) at 4˚C for 30 min, followed by cross-linking with mouse anti-rat IgG (30μg/ml) for various times at 37˚C. (**e)** BMDMs from *Shp-2*$^{fl/fl}$ or MN-*Shp-2*$^{-/-}$ mice were stimulated by OVA-IgG immune complexes (50μg/ml) for 5 or 15 min. OVA-IgG immune complexes were prepared by incubating albumin (Cat.# 02191349.2, MP BIOMEDICALS) with rabbit anti-OVA IgG fraction (Cat.# 0855029, MP BIOMEDICALS) at 1:10 ratio at 37℃for 1 h. **(f**) BMDMs from *Shp-2*$^{fl/fl}$ or MN-*Shp-2*$^{-/-}$ mice were stimulated by heat-induced IgG aggregates (50μg/ml) for 5 or 15 min. IgG aggregates were produced by incubation of mouse IgG (Jackson labs, ImmunoResearch) in borate-buffered saline, pH8.0 at 63℃ for 30 min. Cell lysates were resolved by 10% SDS-PAGE and probed by indicated antibodies. All the experiments were repeated at least twice with similar results, and the representative data are shown.



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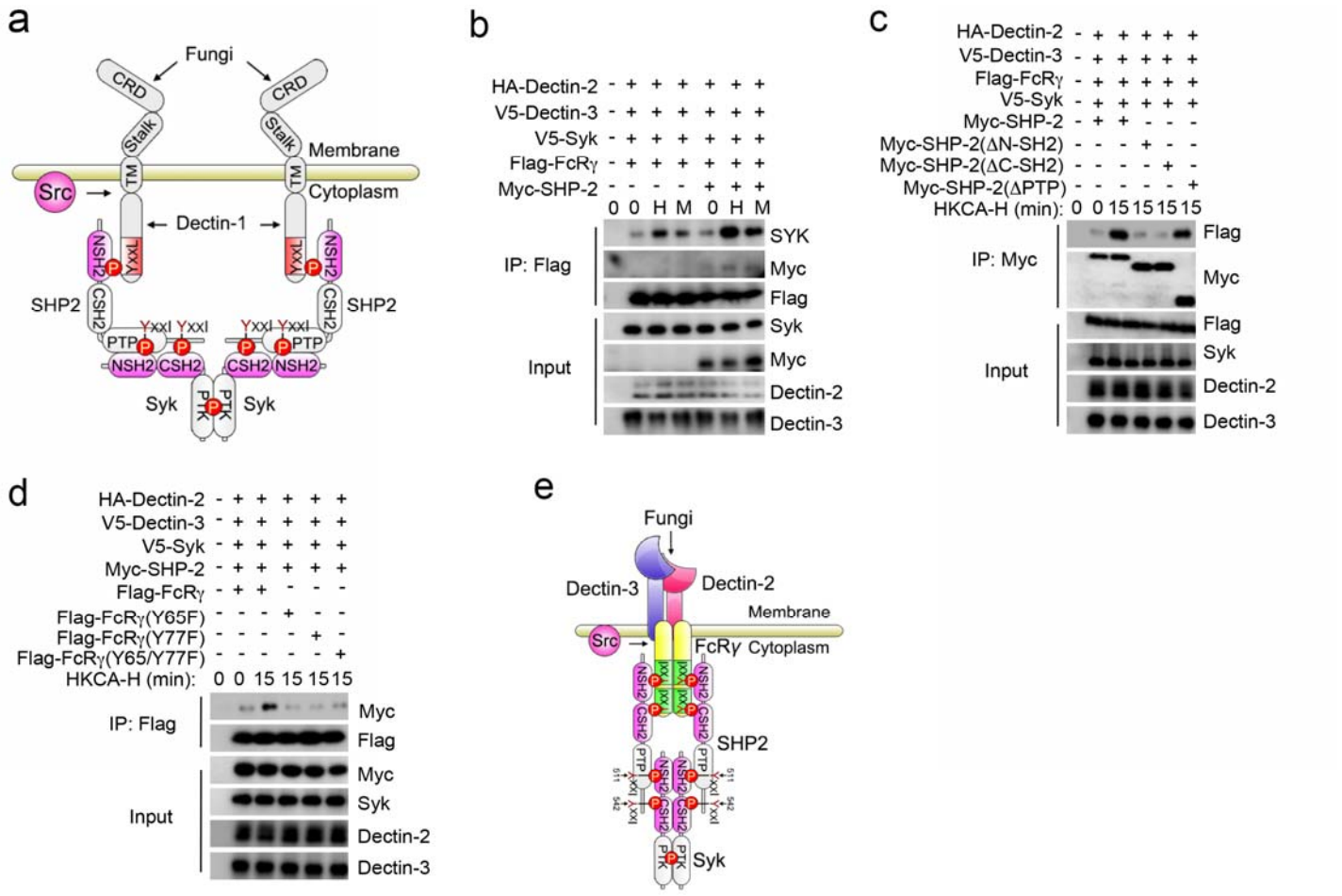
Supplementary Figure 4

SHP-2 recruits Syk to dectin-1 and mediates Syk activation.

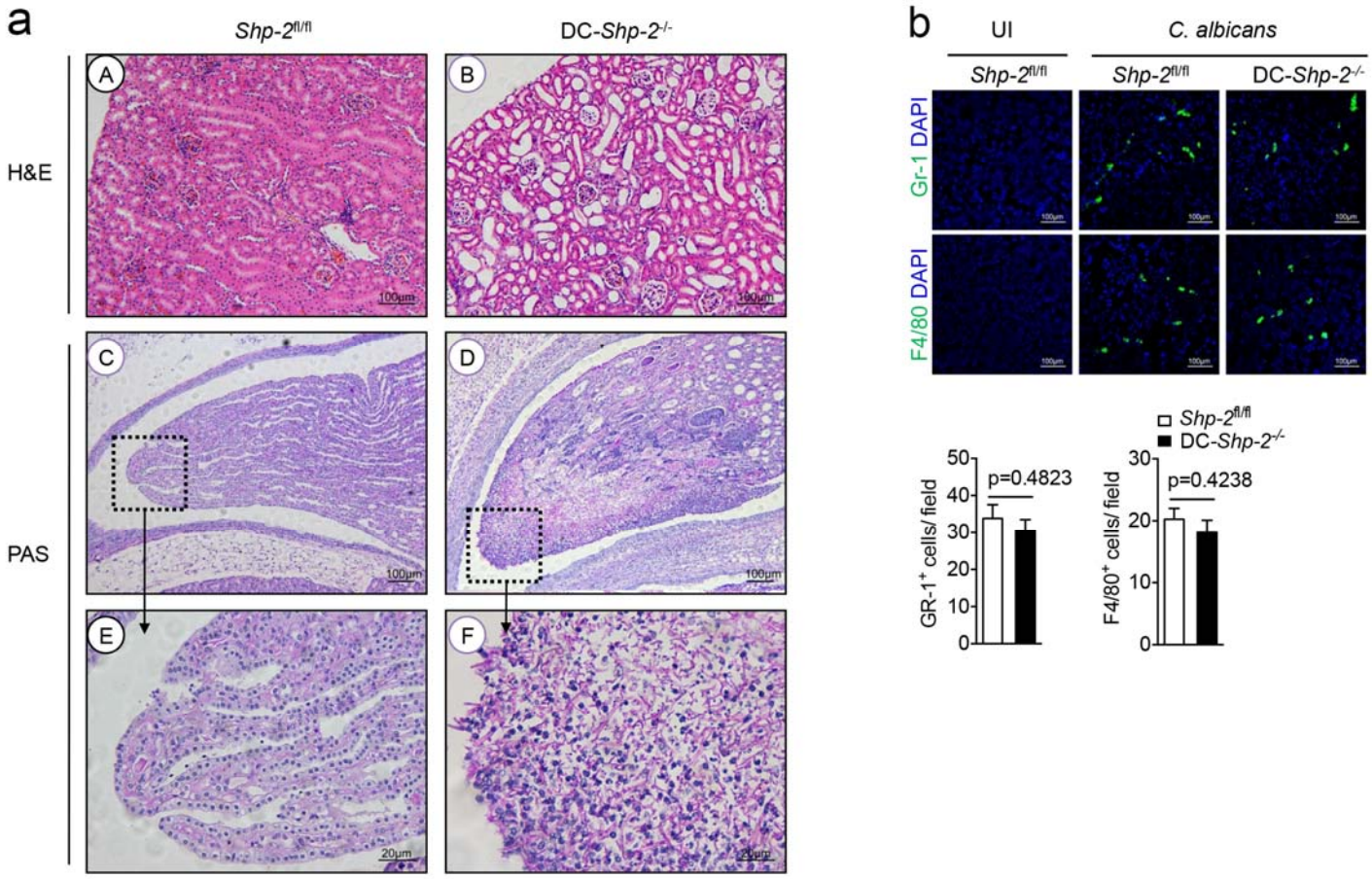
(**a**) HEK293T cells were transiently transfected by various combinations of plasmids expressing V5-Syk, dectin-1 or FLAG-SHP-2. 48 h after transfection, cells were left unstimulated or stimulated by heat-killed *C. albicans* yeast for 15 min. Cell lysates were probed by indicated antibodies. **(b,c)** HEK293T cells were transiently transfected by plasmids expressing Flag-dectin-1, V5-Syk, with or without Myc-SHP-2, Myc-SHP-2 phosphatase-inactive mutant (PD). 48 h after transfection, cells were left unstimulated or stimulated by heat-killed *C. albicans* yeast for 15 min. Cell lysates were immunoprecipitated by anti-Flag and probed by anti-V5, anti-Myc and anti-FLAG, respectively. (**d-f**) MN-*Shp-2*$^{-/-}$ BMDMs were transducted by lentiviral vector pCDH, or pCDH expressing wild type or phosphatase-inactive mutant of SHP-2, respectively. Stable pools of lentiviral-transducted cells were primed by IL-4 (10ng/ml) overnight and then stimulated by Zymd (100μg/ml) for 10 min. BMDMs were fixed by paraformaldehyde and stained by indicated antibodies and DAPI, and fluorescence images were collected by a confocal laser microscope (**d, e**) or fluorescence microscope (**f**). Representative image from at least 20 fields of three different samples for each transducted cell type are shown. The above experiments were repeated twice with similar results.



Supplementary Figure 5

SHP-2 recruits Syk to dectin-1 or FcRγ and mediates Syk activation in CLR signaling.

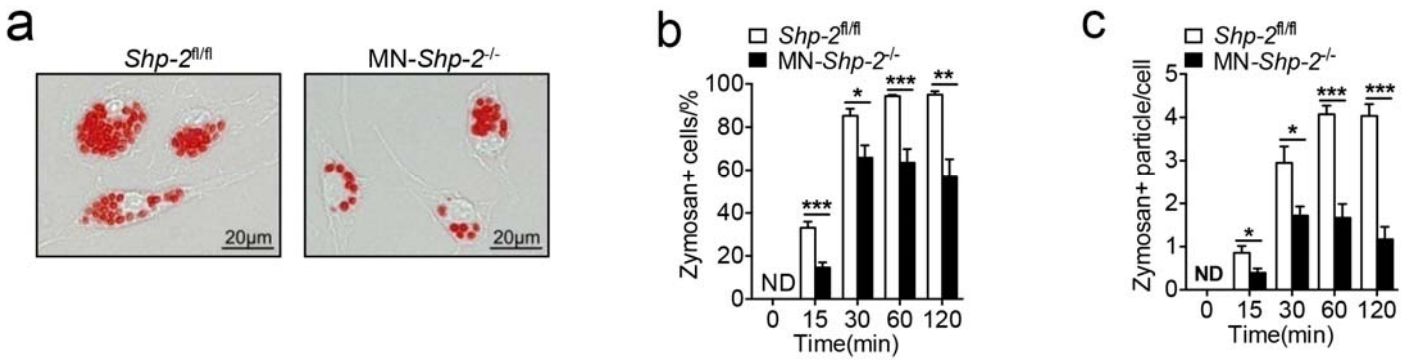
(**a**) Schematic presentation of a proposed model, in which SHP-2 operates as a scaffold protein recruiting Syk to dectin-1 through its N-SH2 and C-terminal ITAM motif. (**b,c**) HEK293T cells were transiently transfected by plasmids expressing HA-dectin-2, V5-dectin-3, Syk, FLAG-FcRγ along with mutants of Myc-SHP-2. 48 h after transfection, cells were left unstimulated or stimulated by mannan (M) or heat-killed *C. albicans* hyphae (H) for 15 min. Cell lysates were immunoprecipitated by anti-Flag and probed by indicated antibodies. These experiments were repeated twice with similar results. (**d**) HEK293T cells were transiently transfected by plasmids expressing HA-dectin-2, V5-dectin-3, Syk, Myc-SHP-2, FLAG-FcRγ or mutants. 48 h after transfection, cells were stimulated by heat-killed *C. albicans* hyphae for 15 min. Cell lysates were immunoprecipitated by anti-Myc and probed by anti-FLAG and anti-Myc sequentially. These experiments were repeated twice with similar results. (**e**) Schematic presentation of a proposed model, in which SHP-2 operates as a scaffold recruiting Syk to FcRγ in dectin-2/3 signaling through its N-terminal SH2 domains and C-terminal ITAM motif.



Supplementary Figure 6

DC-*Shp-2*$^{-/-}$ mice exhibited severer tissue damage and more fungal burden in infected kidneys.

(**a**) Live *C. albicans* SC-5314 (2×10^5 fungal cells in 0.1ml of PBS buffer) were i.v. injected into 6-8 weeks old littermates of distinct genotypes. 5 days after infection, kidneys were harvested and fixed by 10% formalin and paraffin-embedded sections were stained by hematoxylin and eosin or periodic acid-Schiff, respectively. (**b**) *Shp-2*$^{fl/fl}$ and DC-*Shp-2*$^{-/-}$ mice were infected by *C. albicans* SC-5314 (2×10^5 fungal cells per mouse) and kidneys were harvested in 3 days. Kidneys were embedded in OCT and frozen-sections were stained by anti-Gr-1 or anti-F4/80. UI: Uninfected. Representative images were shown and positive cells for each genotype were quantified over 15 fields from three independent samples.



Supplementary Figure 7

MN-*Shp-2*$^{-/-}$ macrophages exhibited impaired phagocytosis of zymosan particles.

(**a-c**) BMDMs from *Shp-2*$^{fl/fl}$ and MN-*Shp-2*$^{-/-}$ mice were incubated with Alexa Fluor 594 labled Zymosan for 2 h (**a**) or various times as indicated (**b, c**). Both zymosan positive cell percentage and zymosan particles per cell were quantified over 6 fields from each genotype. UI: Uninfected. Data are presented as mean ± SEM of two experiments. Note: * p<0.05, ** p<0.01, *** p<0.001.

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Supplementary Table 1

Gene	Clone primers: 5'Forward sequence3'	Clone primers: 3'Reverse sequence5'
SHP-2	ATAGCTAGCATGACATCGCGGAGATGGTT	CCGGAATTCTCATCTGAAACTCCTCTGC TG
SHP-2(ΔN-SH2)	ATAGCTAGCATGACATCGCGGACTCGTAT CAATGC	CCGGAATTCTCATCTGAAACTCCTCTGC TG
Syk	CGCGGATCCGCCACCATGGCGGGAAGTG CTGTGG	CCGCTCGAGCGGTTAACCACGTCGTAG TAG
Syk(ΔN-SH2)	CGCGGATCCGCCACCATGACCGGACCCT TTGAGGACC	CCGCTCGAGCGGTTAACCACGTCGTAG TAG
Dectin-2	CCGGAATTCCCACCATGGTGCAGGAAAG ACAATCC	TGCTCTAGATAGGTAAATCTTCTTCAT TTC
Dectin-3	CGCGGATCCGCCACCATGTGGCTGGAAG AATCCCA	CCGCTCGAGCGCTTCGAGGGCTTCCAA T
FcRγ	ATAGCGGCCGCCTGGGGTGGTTTCTCAT	ATAGCGGCCGCTTACTTATCGTCGTCAT CCTTGTAATCCTGGGGTGGTTTCTCA
Gene	Overlap primers: 5'Forward sequence3'	Overlap primers: 3'Reverse sequence5'
SHP-2(Y542F)	GACATGAATTTACCAATATTAAG	CTTAATATTGGTAAATTCATGTC
SHP-2 (Y511F)	GACATGAATTTACCAATATTAAG	CTTAATATTGGTAAATTCATGTC
SHP-2(ΔC-SH2)	AGACCCTACCTCTGAAACTCGTATCAATG	GCAGCATTGATACGAGTTTCAGAGGTAG G
Dectin-1(Y15F)	CCGGAATTCGCCACCATG AAA TAT CAC TCT CAT ATA G	ATAGCGGCCGCAGCGTAGTCTGGGACG TCG
Syk(ΔC-SH2)	CCACGGCCCATGGTGCACAGATG	CATCTGTGCACCATGGGCCGTGG

Supplementary Table 2

Antibody	Clone	Distributor
Anti-Rabbit- p-PLCγ2 (Tyr759)	#3874	Cell signaling Technology
Anti-Rabbit- PLCγ2	#3872	Cell signaling Technology
Anti-Rabbit- p-Syk (Tyr525/526)	#2710	Cell signaling Technology
Anti-Rabbit- p-PKCδ (Th505)	#9374	Cell signaling Technology
Anti-Rabbit- PKCδ	#9616	Cell signaling Technology
Anti-Rabbit- p-Raf-1 (Ser338)	#9427	Cell signaling Technology
Anti-Rabbit- p-SHP-2 (Tyr542)	#3751	Cell signaling Technology
Anti-Rabbit- p-IκBα (Ser32)	#2859	Cell signaling Technology
Anti-Rabbit- p-p38 (Thr180/Tyr182)	#9211	Cell signaling Technology
Anti-Rabbit- p-JNK (Thr183/Tyr185)	#4668	Cell signaling Technology
Anti-Rabbit- JNK	#9252	Cell signaling Technology
Anti-Rabbit- HA	#3721	Cell signaling Technology
Anti-Rabbit- Src	#2110	Cell signaling Technology
Anti-Rabbit- Erk	SC-93	Santa Cruz Biotechnology
Anti-Mouse- p-ERK	SC-7383	Santa Cruz Biotechnology
Anti-Mouse- p-Tyr	Sc-7020	Santa Cruz Biotechnology
Anti-Rabbit- p38	SC-728	Santa Cruz Biotechnology
Anti-Rabbit- SHP-2	SC-280	Santa Cruz Biotechnology
Anti-Mouse- MYC	SC-40	Santa Cruz Biotechnology
Anti-Rat- dectin1	SC-73897	Santa Cruz Biotechnology
Anti-Rabbi-GAPDH	#AP0063	Bioworld
Anti-Mouse-Syk	ALX-804-480-C100	Enzo Life Sciences
Anti-Rabbit-FLAG	F3165	Sigma
Anti-Mouse-FLAG beads	M2	Sigma
Anti-Rabbit-FcRγ	06-727	Upstate Biotechnology
Anti-Rabbit-Raf-1	AB20484b	Sangon biotech
Anti-Mouse-Mincle	D292-3	MBL International
anti Rat Ly-6G and Ly-6c	550291	BD Biosciences
Anti-Mouse F40/80	14-4801	Ebioscience
Anti-OVA IgG fraction	0855029	MP BIOMEDICALS
Anti-Rat-CD16/32 antibody	2.4G2	BD Biosciences

Supplementary Table 3

Gene	GeneBankID	5'Forward sequence3'	3'Reverse sequence5'
m*IL-17A*	NM_010552	CCAGGGAGAGCTTCATCTGTGT	AAGTCCTTGGCCTCAGTGTTTG
m*IL-17F*	NM_145856	GAAGAAGCAGCCATTGGAGAAA	AGCTGCTACCTCCCTCAGAATG
m*IFN-γ*	NM_008337	GGCACAGTCATTGAAAGCCTAGA	GTCACCATCCTTTTGCCAGTTC
mGAPDH	NM_002046	TGGAGAAACCTGCCAAGTATGA	CTGTTGAAGTCGCAGGAGACAA