CARD9 mediates Dectin-2-induced IKK ubiquitination leading to activation of NF-κB in response to the stimulation by the hyphal form of *Candida albicans*

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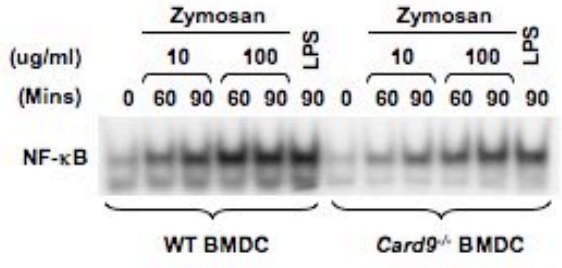
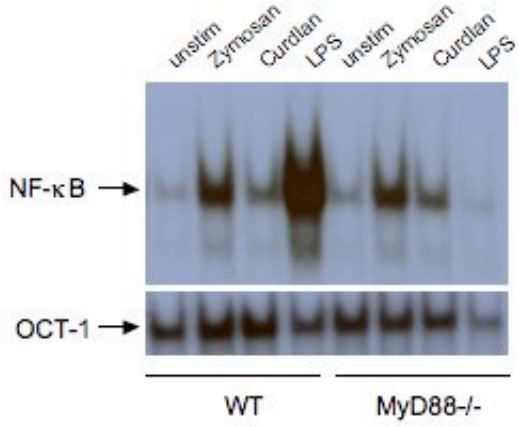
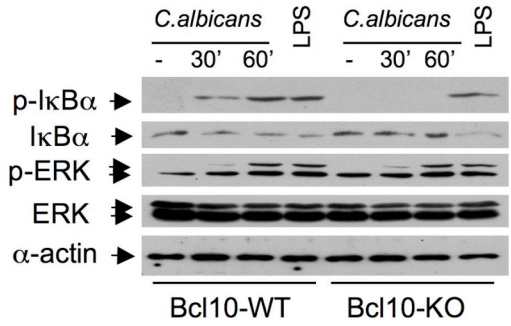
Supplemental Data



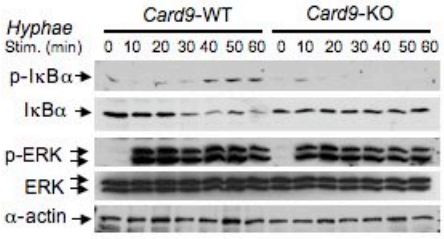
Supplemental Figure 1. Zymosan can induce NF-κB activation in CARD9-deficient dendritic cells. Wild-type or *Card9*$^{-/-}$ Bone marrow derived dendritic cells were stimulated by mock or zymosan at indicated time point and concentration. LPS stimulation (100ng/ml) is used as a positive control. Nuclear extracts were prepared and subjected to EMSA using ^{32}P-labeled NF-κB or Oct-1 as probes.



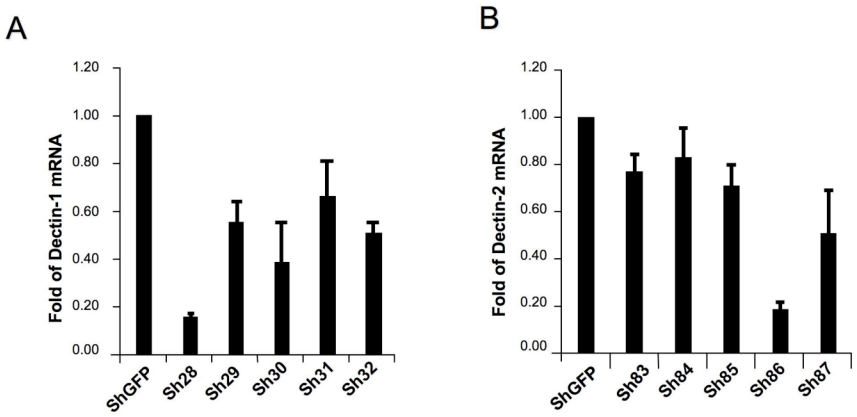
Supplemental Figure 2. Zymosan- and Curdlan-induced NF-κB activation is independent on MyD88. Wild-type or *MyD88*$^{-/-}$ Bone marrow derived macrophages were stimulated by zymosan, Curdlan, or LPS (100ng/ml) for one hour. Nuclear extracts were prepared and subjected to EMSA using ^{32}P-labeled NF-κB or Oct-1 as probes.



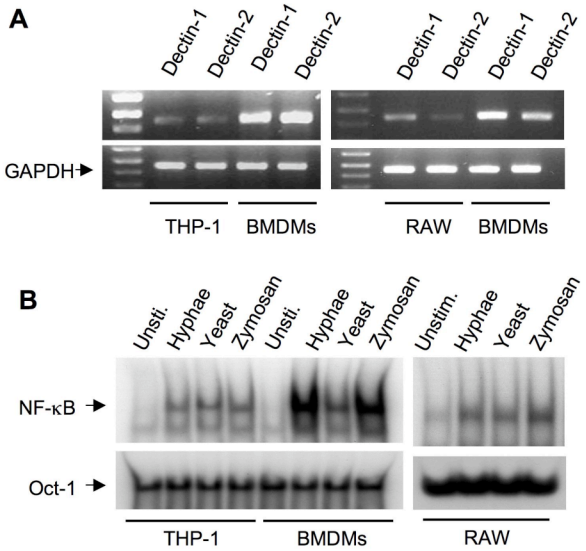
Supplemental Figure 3. Bcl10 is required for *C.albicans*-induced IκBα phosphorylation and degradation. Wild-type (WT) and Bcl10$^{-/-}$ (KO) BMDMs were stimulated with *C.albicans* (MOI=1) and LPS (100ng/ml) for indicated time. Cell lysates were prepared and subjected to immunoblotting analysis using indicated antibodies.



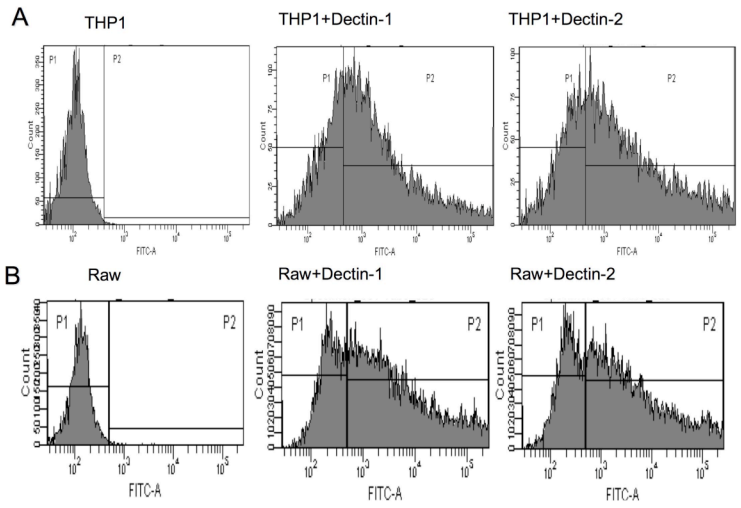
Supplemental Figure 4. Wild-type and *Card9*$^{-/-}$ BMDMs stimulated with hyphae form of *C.albicans* at MOI=1 for indicated time points. Cell lysates from these samples were subjected to immunoblotting analysis using indicated antibodies.



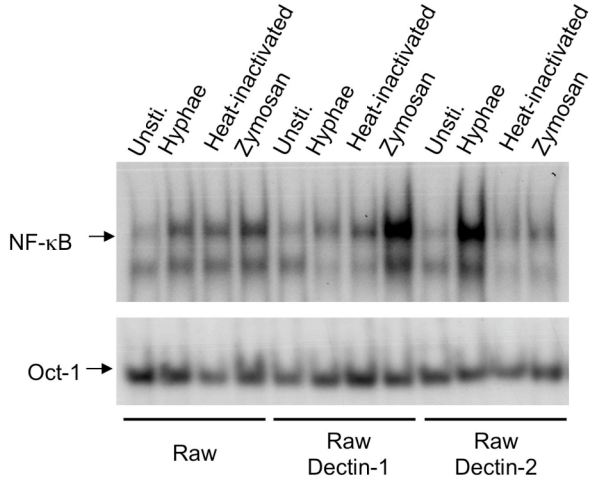
Supplemental Figure 5. Knockdown efficiency of lentivirus-encoded shRNA for Dectin-1 and Dectin-2. Quantification of Dectin-1 (A) and Dectin-2 (B) mRNA level in BMDM infected with indicated shRNAs for Dectin-1 (A) or Dectin-2 (B), or shRNA for GFP by quantitative real-time PCR. The lentiviral vector encoding sh28 and sh86 were later used for knocking down Dectin-1 and Dectin-2, respectively.



Supplemental Figure 6. The expression level of Dectin-1 or Dectin-2 in cells affects *C.albicans*- or Zymosan-induced NF-κB activation. (A) The expression level of Dectin-1 and Dectin-2 in THP-1, RAW264.7, and BMDMs was detected by RT-PCR, GAPDH as a control. (B) THP-1, RAW264.7, and BMDMs were stimulated with hyphae, yeast and zymosan for 1 hour. Nuclear extracts from these cells were prepared and subjected to EMSA using ^{32}P-labeled NF-κB or Oct-1 probe**.**



Supplemental Figure 7. Infection efficiency in THP-1 and RAW264.7 cells. (A) THP-1 or (B) RAW264.7 cells were infected with lentivirus encoding Dectin-1 or Dectin-2 together with lentivirus-encoding GFP. GFP-positive cells were indicated by X-axis in flow cytometry.



Supplementary Figure 8. Dectin-2-expression in RAW264.7 cells enhanced Hyphae-induced NF-κB activation. Raw264.7 cells stably infected with lentivirus-encoding Dectin-1, Dectin-2 or vector alone were stimulated with hyphae, yeast, and zymosan for 1 hour. Nuclear extracts from these cells were prepared and subjected to EMSA using ^{32}P-labeled NF-κB or Oct-1 probe**.**