SUPPLEMENTAL MATERIAL

Robinson et al., http://www.jem.org/cgi/content/full/jem.20082818/DC1

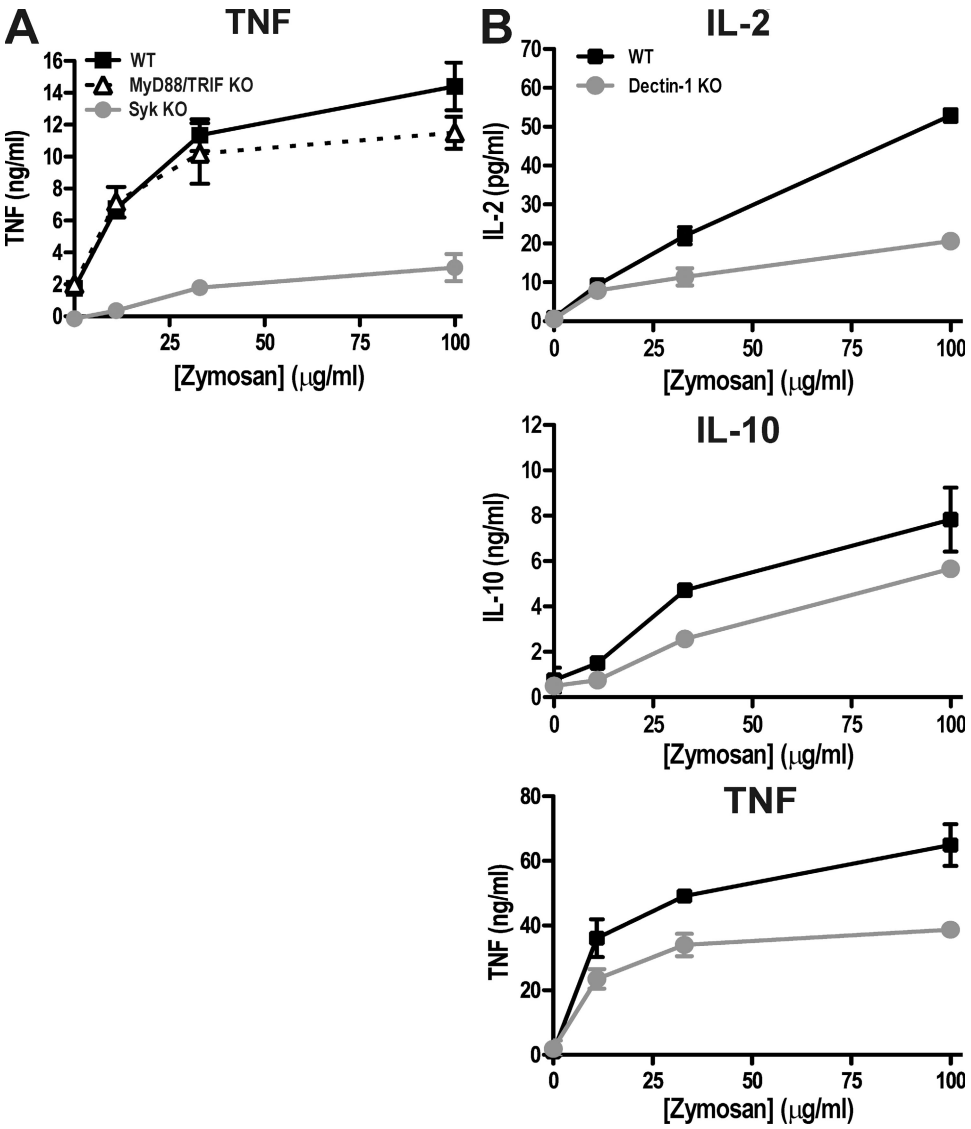

Figure S1. The contribution of Dectin-1, Syk, and TLR signaling to cytokine induction by zymosan. (A) BMDCs from C57BL/6 wild-type (WT, black squares), *Myd88*$^{-/-}$/*Trif*$^{-/-}$ (MyD88/TRIF DKO, white triangles), or *Syk*$^{-/-}$ chimeric (Syk KO, gray circles) mice were stimulated with increasing doses of zymosan. (B) As in A, comparing BMDCs from C57BL/6 wild-type (WT, black squares) and *Clec7a*$^{-/-}$ chimeric (Dectin-1 KO, gray circles) mice. Cytokine levels in the supernatants were quantified after overnight incubation. Data are means ± SD of duplicate wells and are representative of at least three independent experiments.

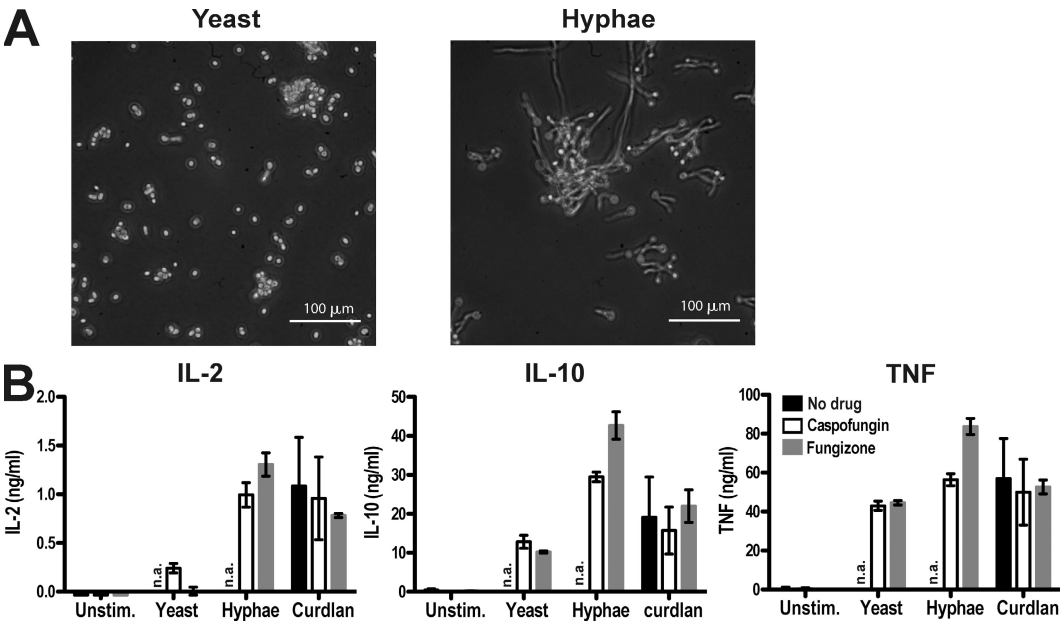

Figure S2. Antifungal drugs have minor effects on the Syk-dependent response to *C. albicans* or to curdlan. (A) Morphology of heat-killed yeast and heat-killed hyphae. (B) 10^5 BMDCs from wild-type mice were stimulated with 10^5 live *C. albicans* yeast or hyphae, or with 100 µg/ml curdlan. 50ng/ml caspofungin or 2.5 µg/ml fungizone were added 2 h later, and cytokine levels in the supernatants were measured after overnight incubation. Data are means ± SD of duplicate wells and are representative of three independent experiments. n.a., not applicable.

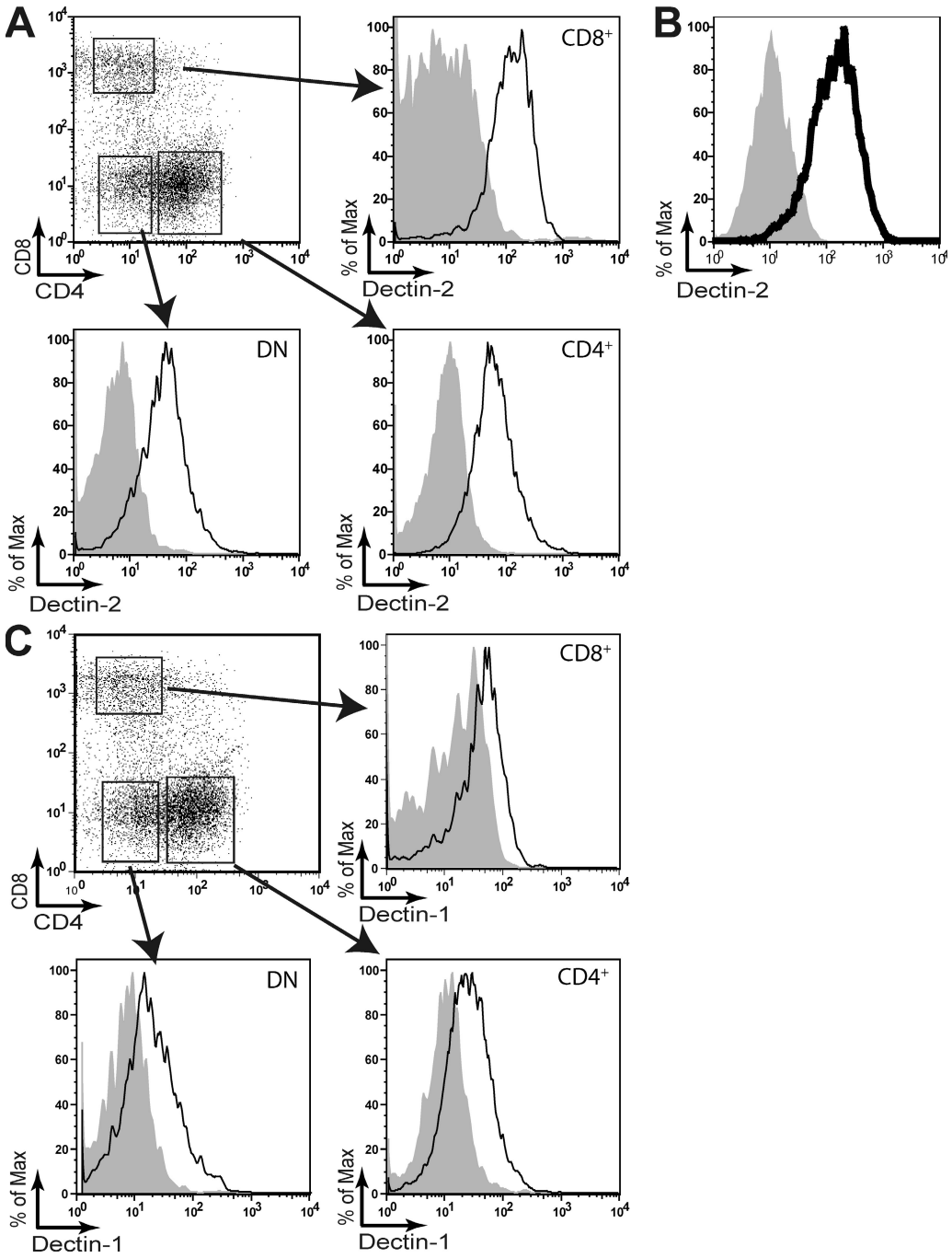

Figure S3. Dectin-2 expression by DCs. (A) CD11c-enriched splenocytes were stained for CD11c, CD4, CD8, and anti–Dectin-2 or isotype-matched control. (top left) Gating of CD11c^{hi} conventional DC populations into CD4$^+$, CD8$^+$, and double negative DCs. The other panels are histograms of anti–Dectin-2 (open) or isotype control (shaded) staining in the gated subpopulations. (B) Staining of BMDCs with anti–Dectin-2 (open histogram) or isotype control (shaded histogram). (C) As in A, except that cells were stained with anti–Dectin-1. Data are representative of three (B) or two (A and C) independent experiments.

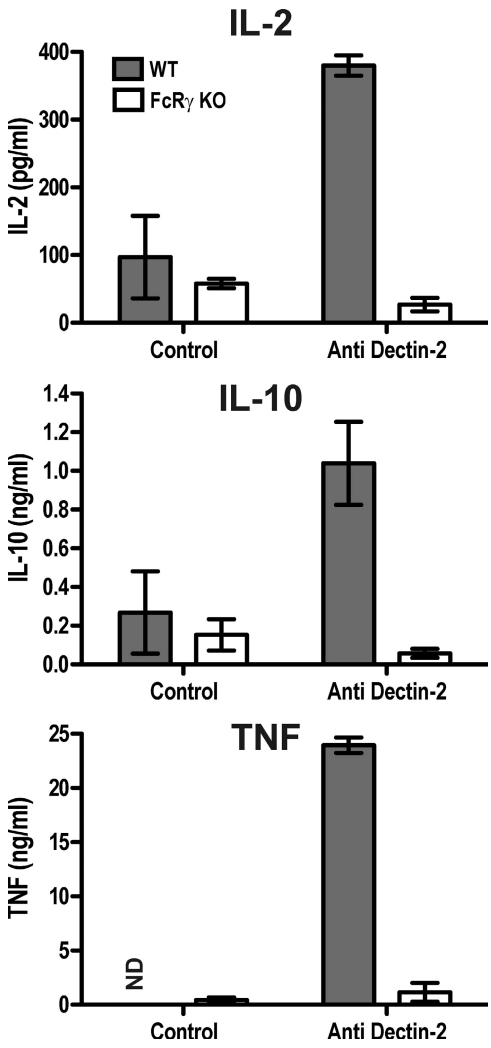

Figure S4. Activation of DCs by plate-immobilized anti–Dectin-2 Fab is dependent on FcRγ chain. BMDCs from C57BL/6 (WT) or *Fcer1g*$^{-/-}$ (FcRγ chain KO) mice were stimulated overnight with plated anti–Dectin-2 or control Fab, and cytokine levels in the supernatants were measured. Data are means ± SD of duplicate wells and are representative of three independent experiments. ND, not detectable.

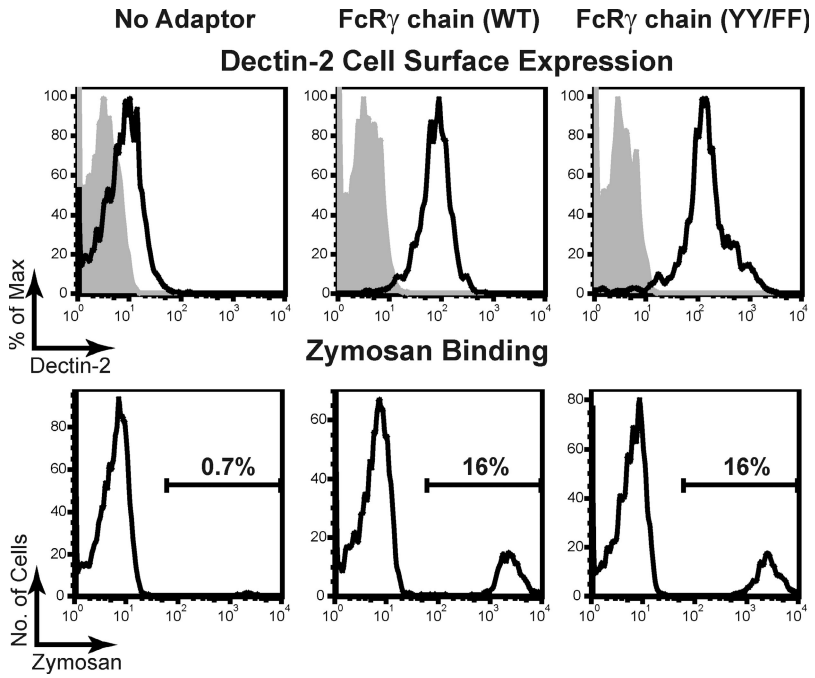

Figure S5. Dectin-2 requires FcRγ chain but not ITAM signaling for cell-surface expression and zymosan binding. B3Z cells (No Adaptor) or sublines derived by transduction with wild-type FcRγ chain (FcRγ chain (WT)) or a signaling-deficient mutant of FcRγ chain (FcRγ chain (YY/FF)) were subsequently transduced with Dectin-2–IRES–EGFP. (top) Anti–Dectin-2 staining of GFP-positive cell lines (open histogram) as compared with staining with an isotype-matched control mAb (shaded histogram). (bottom) Cy5-zymosan binding to the above cell lines. Data are representative of two independent experiments.

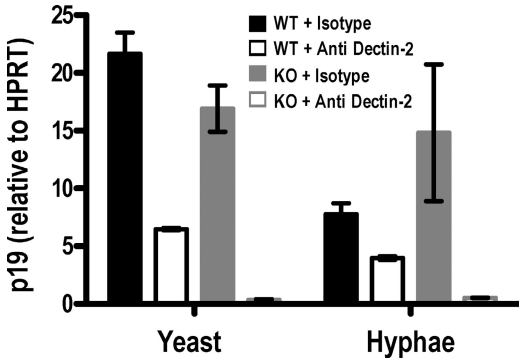

Figure S6. Dectin-1 and -2 mediate *C. albicans* induction of IL-23. 4×10^5 BMDCs from wild-type C57BL/6 (WT) or *Clec7a*$^{-/-}$ (Dectin-1 KO) mice were treated with 10 µg/ml anti–Dectin-2 or isotype control for 2 h before stimulation with 4×10^5 live *C. albicans* yeast or hyphae. IL-23 p19 mRNA was quantitated by quantitative PCR after 3 h. Fungizone was added after 2 h for the final 1 h of stimulation. Data are means ± SD of duplicate wells and are representative of two independent experiments.

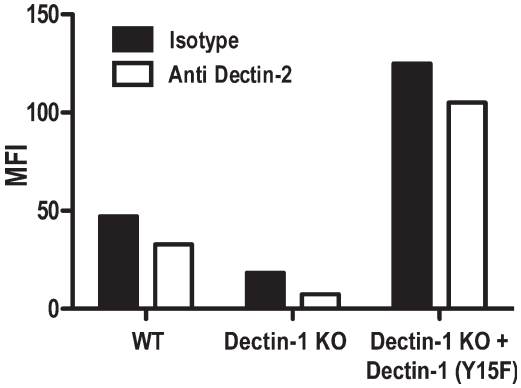

Figure S7. The contribution of Dectin-1 and -2 to binding of zymosan. BMDCs from *Clec7a*$^{-/-}$ chimeric mice were transduced with signaling-deficient Dectin-1 (Dectin-1 KO + Dectin-1 (Y15F)) or mock transduced (Dectin-1 KO). These cells and mock-transduced C57BL/6 BMDCs (WT) were treated with 10 µg/ml anti–Dectin-2 or isotype-matched control mAb for 2 h before testing for binding of Cy5-zymosan (added at 33 µg/ml). Data are mean fluorescence intensity (MFI) and are representative of at least three independent experiments.

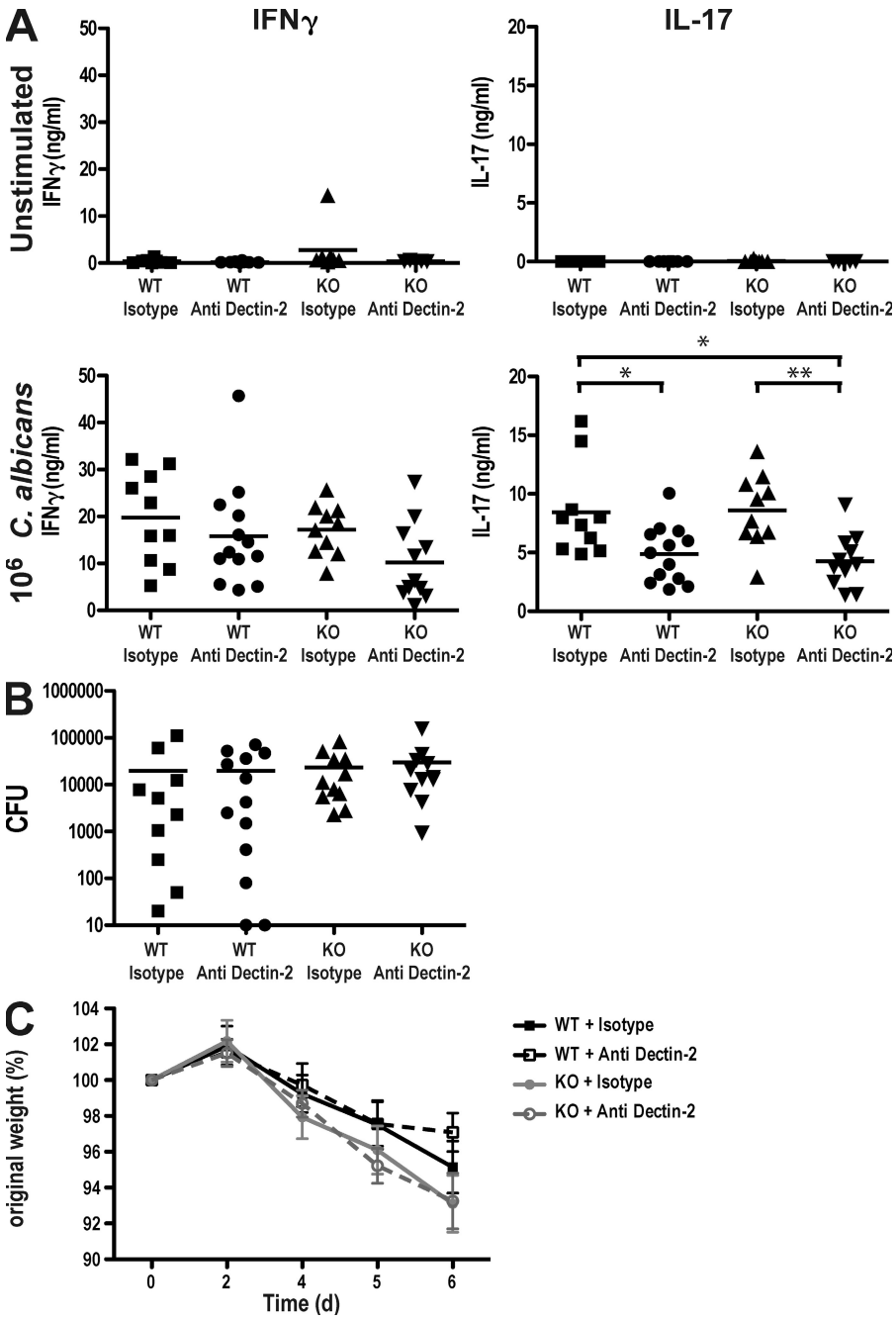

Figure S8. Dectin-2 is required for IL-17 production by restimulated splenocytes but not for containing early renal fungal burden in systemic *C. albicans* infection. Wild-type 129/Sv (WT) or *Clec7a*$^{-/-}$ (Dectin-1 KO) mice were treated with anti–Dectin-2 or isotype-matched control mAb and infected with *C. albicans* i.v. After 7 d, mice were sacrificed. (A) splenocytes were restimulated with 0 or 10^6 heat-killed *C. albicans* for 2 d, and IFN-γ and IL-17 cytokines were measured in the supernatants. (B and C) Kidney fungal burden (B) and percentage of original weight at time of sacrifice (C). The data in A–C are pooled from two independent experiments, and each symbol represents the mean of triplicate stimulations from an individual mouse. Statistically different groups are indicated (*, 0.01 < P < 0.05; and **, 0.001 < P < 0.01).