Supplemental Information

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EXTENDED EXPERIMENTAL PROCEDURES

BMM and BMDC cultures and ELISA Assays

BMMs and BMDCs were prepared from C57BL/6 or gene-deficient mice using L929 cell (M-CSF source) or p815 cell (GM-CSF source) conditioned medium (Hohl et al., 2008). For BMDC preparations, $CD11c^+$ cells were further enriched using Miltenyi magnetic bead selection.

A. fumigatus germlings and hyphae were grown in 96-well format for 7 hr or 18 hr, respectively, starting from 150,000 or 50,000 resting conidia and UV inactivated prior to addition of 150,000 BMMs or BMDCs for 18h stimulation experiments. Live conidia were added to BMDCs at a 10:1 ratio with 0.5 μg/ml voriconazole (Pfizer). Commercially available kits from eBioscience or R&D Systems were used to measure cytokine levels in culture supernatants or in clarified tissue and organ homogenates from infected mice.

In Vitro Neutrophil Conidial Uptake Assay

For fungal uptake assays, 2×10^6 BM cells were incubated with 2×10^6 FLARE conidia in 2 ml RP10 at 37°C. The frequency of $AF633^+$ neutrophils was assessed by flow cytometry at t = 8 hr. Pilot experiments showed that BM neutrophil conidial uptake reached a plateau at this time point. No neutrophil conidial uptake was observed when co-incubations were performed at 4°C.

SUPPLEMENTAL REFERENCE

Hohl, T. M., Feldmesser, M., Perlin, D. S., and E. G. Pamer. (2008). Caspofungin modulates inflammatory responses to *Aspergillus fumigatus* through stage-specific effects on fungal β-glucan exposure. J. Infect Dis. *198*, 176–185.

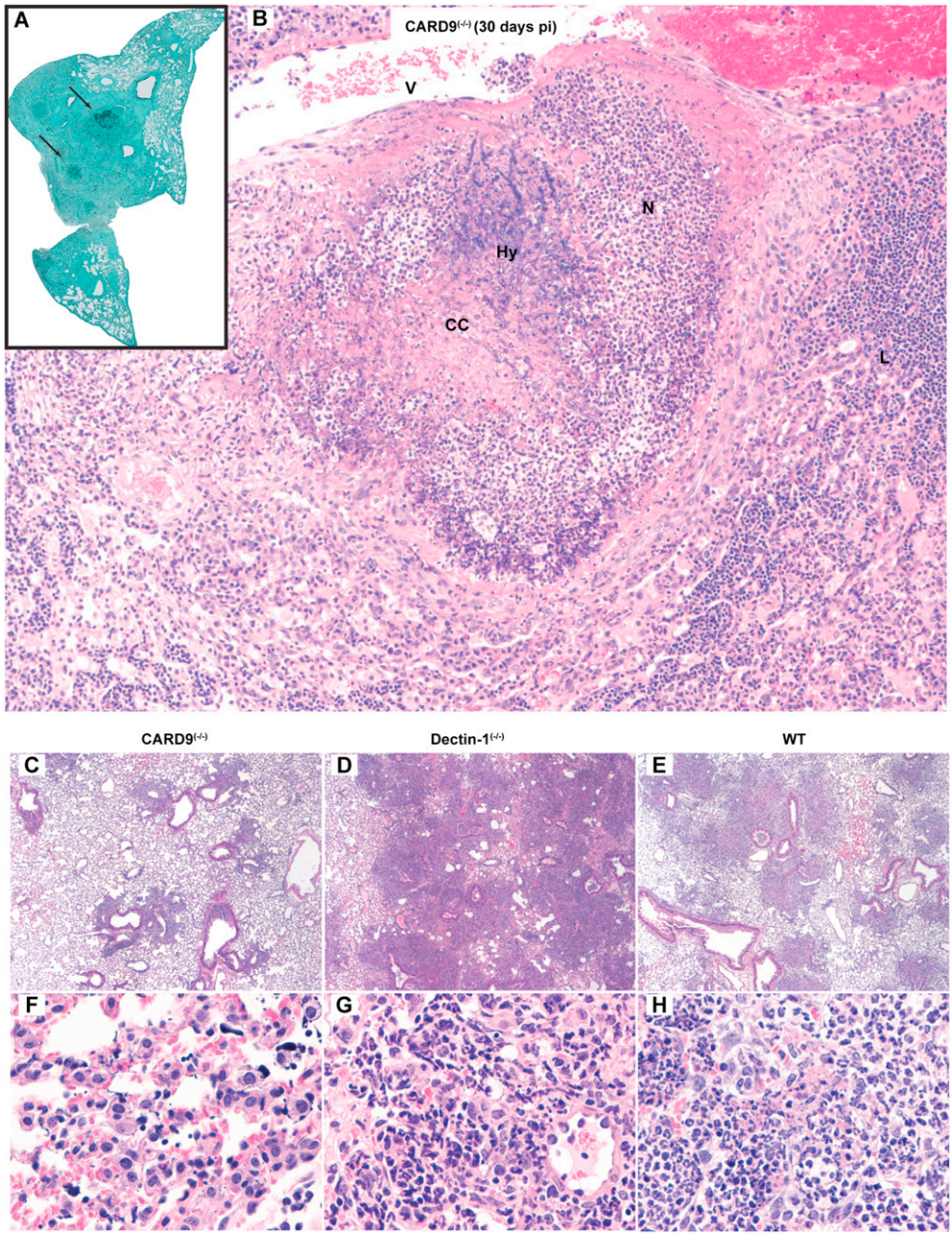

Figure S1. CARD9 and Lung Histopathology during Respiratory Fungal Infection, Related to Figure 4

(A–H) The micrographs show representative GAS (A) and H&E (B–H) stained lung sections from (A–C and F) CARD9$^{(-/-)}$, (D and G) Dectin-1$^{(-/-)}$, and (E and H) WT mice, 3 (C–H) or 30 (A and B) days p.i. with 8 × 10^7 *A. fumigatus* conidia. Micrographs are at (A) 2.5x, (B) 10x, (C–E) 4x, and, (F–H) 60x magnification.

(A) The arrows indicate fungal lesions in GAS-stained lung sections.

(B) The micrograph depicts an example of these pyogranulomatous lesions in CARD9$^{(-/-)}$ mice, consisting of a central core of caseous necrosis (CC) and fungal hyphae (Hy) that are surrounded by neutrophils (N). At the periphery of the lesion, fibrous connective tissue, lymphocytes and plasma cells (L) are present. A blood vessel is indicated by (V).

(C and F) Lung sections from CARD9$^{(-/-)}$ mice, euthanized 3 days p.i., display mild inflammatory cell infiltrates composed of neutrophils and macrophages surrounding bronchioles and vessels, and extending into alveolar spaces. The affected areas in Dectin-1$^{(-/-)}$ and WT lungs reveal a more severe and coalescing inflammatory infiltrate in WT mice (E and H), and in particular in Dectin-1$^{(-/-)}$ (D and G), consistent with lung neutrophil recruitment data (Figure 4C). Representative lung sections are from 2 experiments with 3 mice per group.

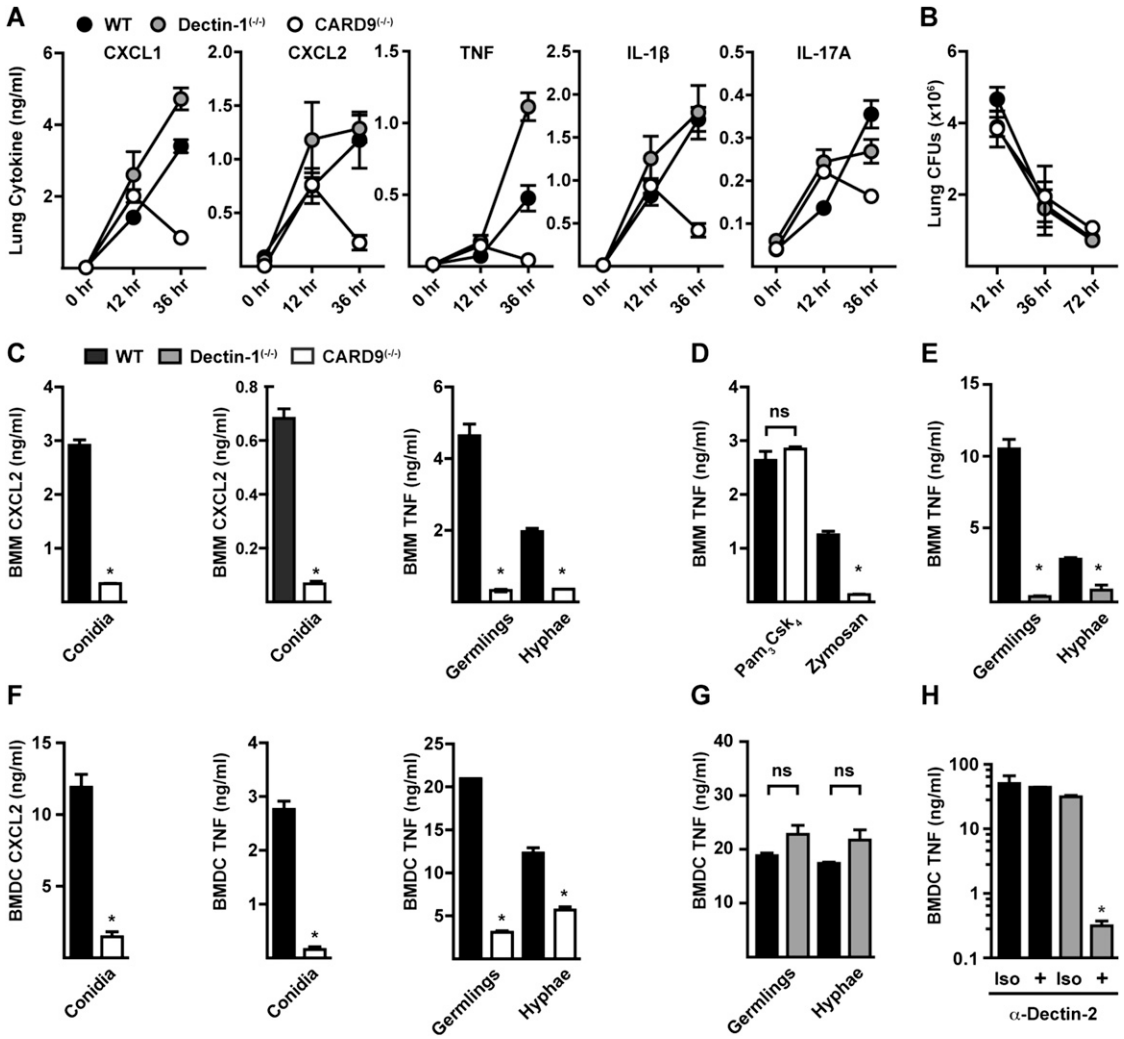

Figure S2. *A. fumigatus* Activates Multiple CARD9-Coupled Receptors, Related to Figure 4

(A and B) WT (black circles), Dectin-1$^{(-/-)}$ (gray circles) and CARD9$^{(-/-)}$ (white circles) mice were infected with 3 × 10^7 conidia and euthanized 12 hr and 36 hr p.i. (A) The graphs show average lung cytokine levels ± SEM from a representative experiment (n = 4-6 mice per genotype), and (B) average (±SEM) lung fungal CFU from 8-14 mice of each genotype per time point, pooled from 2-3 experiment.

(C–H) WT (C57BL/6; black bars), Dectin-1$^{(-/-)}$ (gray bars) and CARD9$^{(-/-)}$ (white bars) BMMs (C–E) or BMDCs (F–H) were incubated with (C–G) no Ab or (H) α-Dectin-2 or IgG2a isotype control mAb (10 μg/ml) and stimulated with (C and E–H) *A. fumigatus* conidia (moi 1:5), germlings (1:1), hyphae (3:1), (D) NaClO-oxidized zymosan (10 μg/ml) or Pam_3Csk_4 (1 μg/ml). The graphs show the average CXCL2 or TNF levels (±SEM) in culture supernatants from a representative experiment (performed 2-5 times) with 3-4 replicates per condition.

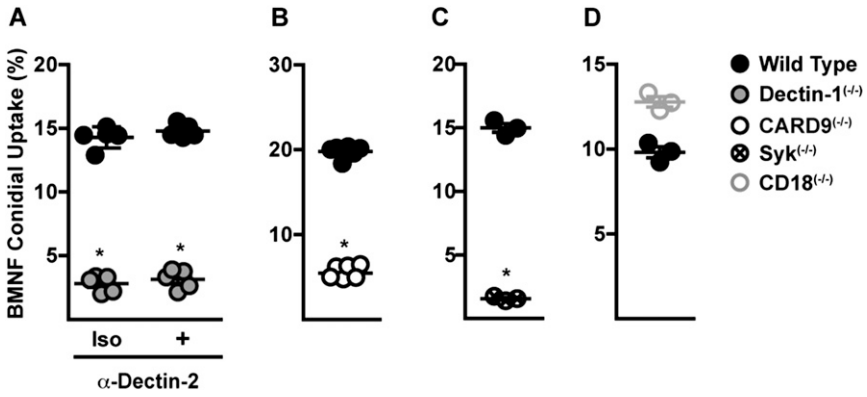

Figure S3. Dectin-1, CARD9, and Syk Mediate Neutrophil Conidial Uptake In Vitro, Related to Figure 4

(A–D) The scatterplots show the average (±SEM) fungal uptake by WT, Dectin-1$^{(-/-)}$, CARD9$^{(-/-)}$, Syk$^{(-/-)}$ and CD18$^{(-/-)}$ BM neutrophils at a moi of 1. (A) Anti-Dectin-2 or IgG2a isotype control antibody was included in co-incubations at a concentration of 10 μg/ml. Results representative of 2-5 (A, B, and D) or 1 (C) experiment(s) with 3-6 replicates per condition.

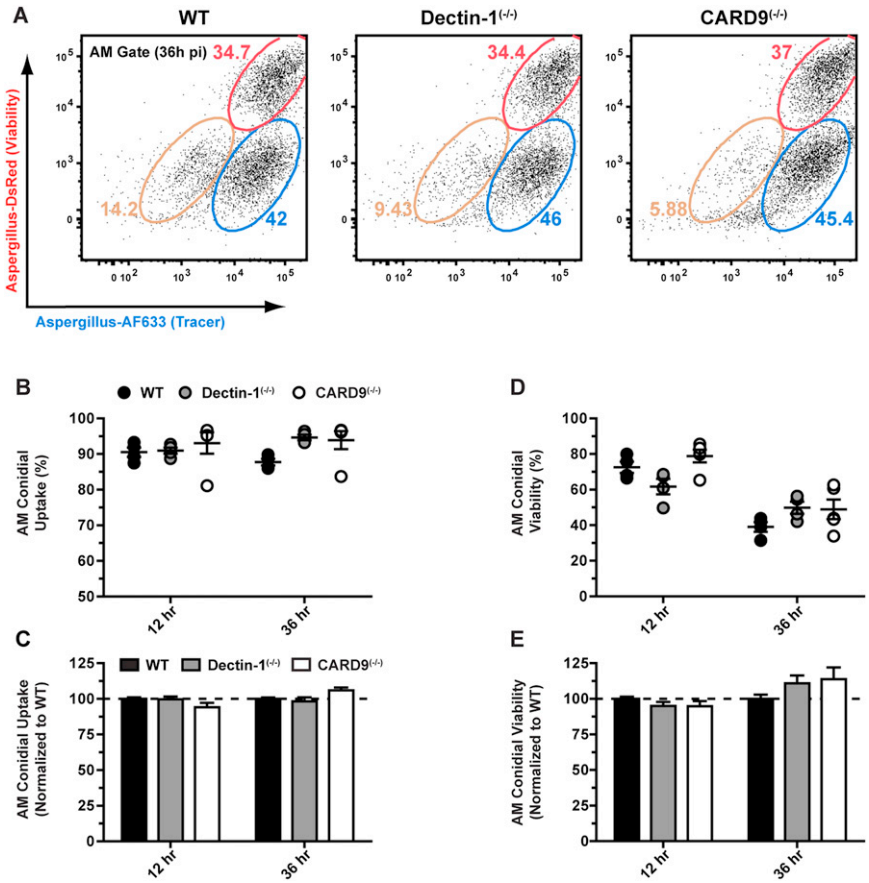

Figure S4. CARD9 and AM Function during Respiratory Fungal Infection, Related to Figure 4

(A–E) WT, Dectin-1$^{(-/-)}$ and CARD9$^{(-/-)}$ mice were infected with 3 × 10^7 FLARE conidia.

(A) Flow plots of AMs analyzed for DsRed and AF633 fluorescence illustrate the frequencies of bystander AMs (tan gates) and AMs that contain live (red gates) or killed conidia (blue gates).

(B and D) The scatterplots show conidial uptake and viability in WT (black circles), Dectin-1$^{(-/-)}$ (gray circles), and CARD9$^{(-/-)}$ (white circles) AMs at 12 hr or 36 hr p.i. from a representative experiment.

(C and E) The plots show the normalized AM conidial uptake and viability using data pooled from 4 (12 hr p.i.) and 3 (36 hr p.i.) experiments. Data are represented as mean ± SEM.

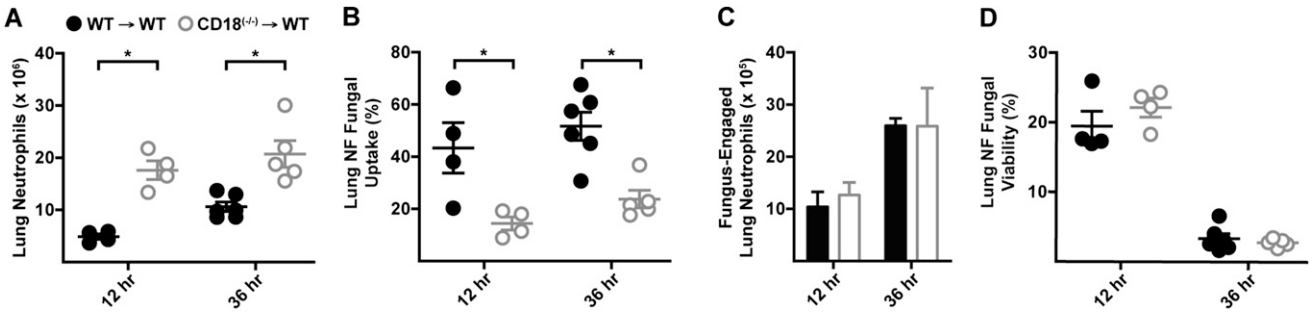

Figure S5. CD18 and Neutrophil Function during Respiratory Fungal Infection, Related to Figure 5

$CD18^{(-/-)}$ → WT (white circles or bars with gray border) and WT → WT (black circles or bars) mice were infected with 3×10^7 FLARE conidia and analyzed 12 hr and 36 hr p.i.

(A–D) The graphs show (A) lung neutrophil recruitment, (B) lung neutrophil conidial uptake, (C) total fungus-engaged neutrophils, and (D) conidial viability in lung neutrophils for $CD18^{(-/-)}$ → WT and WT → WT mice using data pooled from 2 independent experiments.