SUPPLEMENTARY FIGURE/TABLE LEDGENDS

Supplementary Figure 1: Phenotype of CD1d-/- mice and IL-17 secretion by iNKT cells following infection with *A. fumigatus*. WT or CD1d-/- B6 mice were infected i.t. with $4x10^7$ live *A. fumigatus* conidia then sacrificed 2 days post-infection or at the indicated timepoint. (**a**) Lung sections were analyzed by H&E staining and (**b**) inflammation was quantified by morphometric analysis. Bars, mean ± SEM **(c)** Absolute numbers of neutrophils ($CD45^+$ $CD11c^-$ $CD11b^+$ $Ly6C^+$ $Ly6G^+$), monocytes ($CD45^+$ $CD11c^-$ $CD11b^+$ $Ly6C^+$ $Ly6G^-$), macro-phages ($CD45^+$ $CD11c^+$ $MHCII^+$ $FITC^+$ PE^+) and DC subsets ($CD45^+$ $CD11c^+$ $MHCII^+$ $CD103^+$ $CD11b^-$ or $CD45^+$ $CD11c^+$ $MHCII^+$ $CD103^+$ $CD11b^+$) were determined by flow cytometry. **(d)** %IL-17-secreting iNKT cells in the lung as measured by flow cytometry. Bars, mean ± SEM**,** data pooled from 3 independent experiments, n=6-9 mice.

Supplementary Figure 2: *A. fumigatus* activates iNKT cells *in vitro*. iNKT cells of the indicated lines were co-cultured with sorted WT or CD1d-/- BMDCs with 2-5μg/mL of *A. fumigatus*. IFN-γ secretion was measured in the culture supernatant by ELISA. Bars, mean ± SEM, data are representative of at least 3 independent experiments.

SUPPLEMENTARY TABLE 1

	Live cells		CD19- lymphocytes		
	Non-lymphoid	Lymphoid	TCR-β+ tet+	TCR-β+ tet-	TCR-β- tet-
Day 3	61.5 (±4.3)	38.5 (±4.3)	5.9 (±0.8)	18.7 (±2.2)	74.4 (±2.8)
Day 6	69.8 (±2.5)	30.2 (±2.5)	7.0 (±1.4)	47.5 (±5.8)	44.6 (±5.1)

Phenotype of IFN-γ-secreting cells in the lungs of *A. fumigatus*-infected mice. The surface phenotype and FSC/SSC characteritics of IFN-γ^+ live cells (left) or $CD45^+CD19^-$ lymphocytes (right) were analyzed by flow cytometry to determine the relative contribution of different cell type to the pulmonary IFN-γ response following *A. fumigatus* infection. Data was obtained from 4 pooled experiments, n=9-12 mice per timepoint.

SUPPLEMENTARY TABLE 2

Glycosyl Residue	Mol %
Arabinose	-
Rhamnose	-
Fucose	-
Xylose	-
Glucuronic Acid	-
Galacturonic Acid	-
Mannose	1.7
Galactose	2.4
Glucose	49.7
N-Acetyl Galactosamine	-
N-Acetyl Glucosamine	46.2
N-Acetyl Mannosamine	-

Supplementary Table: Sugar composition of the acid/base reflux pellet. Glycosyl compositional analysis of alkali- and acid-insoluble material from *A. fumigatus* hyphae using per-*O*-trimethylsilyl (TMS) derivatized and Gas Chromatography.

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Reagents and antibodies. Anti-mouse Dectin-1 mAb was purchased from Cell Sciences, anti-mouse CD45, CD19, TCRβ, CD103, CD11b, IL-12p70, IL-12p40 antibodies from Ebioscience, CD69, CD11c, CD11b, Ly6C, Ly6G, IFN-γ, Sreptavidin-HRP, Hamster IgG1 and FcR from BD Biosciences, CD45 and MHC Class II from Biolegend, and CD1d tetramer was provided by the NIH. Recombinant murine GM-CSF and IL-4 were purchased from Peprotech, DNAse I from Roche, Type IV collagenase from Worthington Biochemicals, endotoxin-free PBS from Invitrogen, Silica TLC plates from Merck, Sabouraud Dextrose (SD) and Yeast Peptone Dextrose (TPD) broth from Sigma, Sabouraud Dextrose Agar (SDA) slants and Potato Dextrose Agar (PDA) plates from Remel. LPS (*S. abortus equii*) and chitin (crab shell) were purchased from Sigma, CpG ODN1826 and zymosan from Invivogen, zymolyase from Zymo Research and curdlan (*A. biobar*) from Wako Chemicals. Galactomannan was precipitated from *A.f.* hyphal culture supernatants (Latge et al., 1994). Scleroglucan (*S. rolfsii*) was provided by Gary Ostroff. The fungal strains used were *A.f.* 293, *H.c.* G186R, *C.n.* KN99α and *C.a.* (CA6).

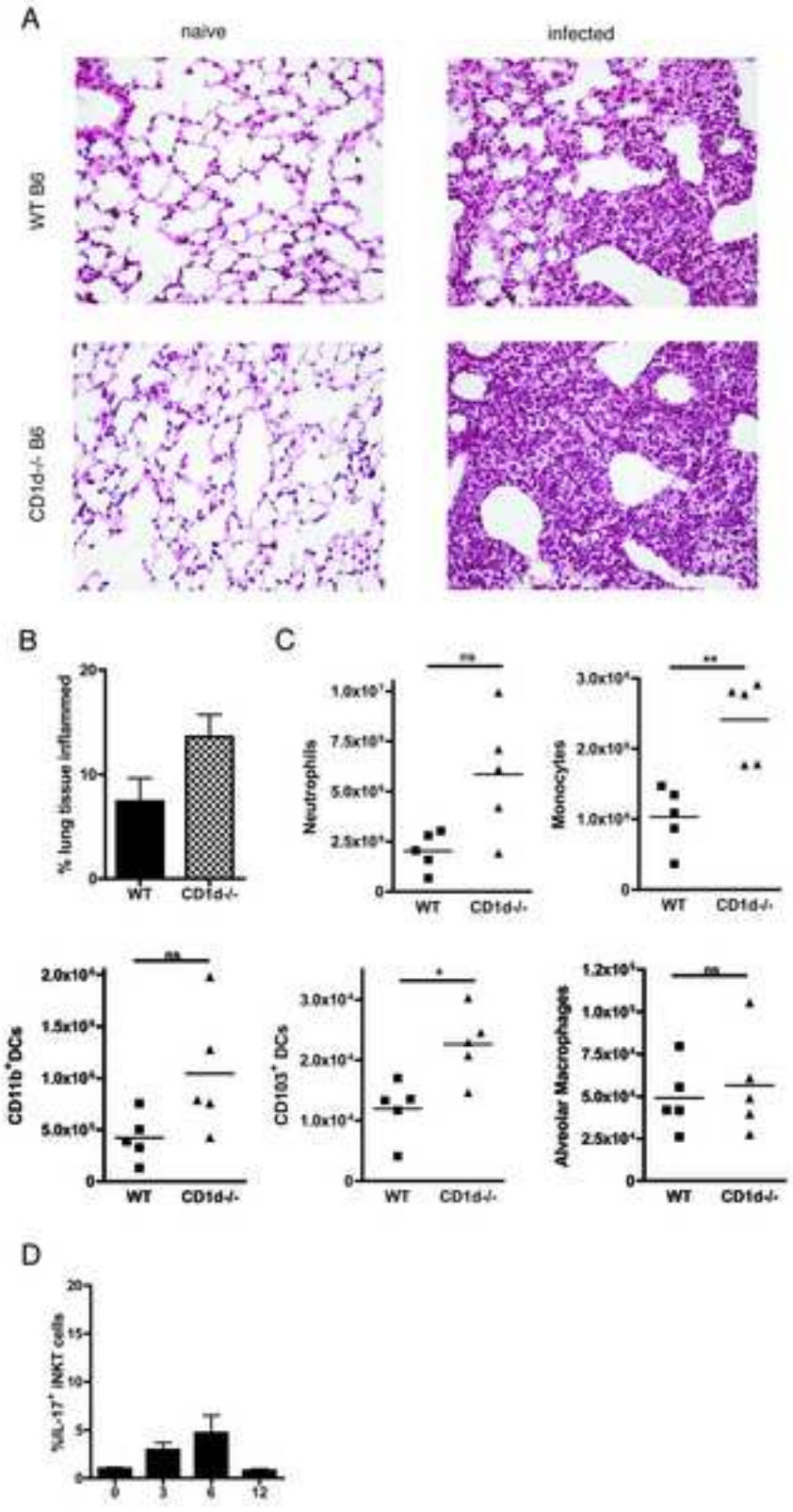
Cells. DCs were derived by culturing bone marrow cells for 5-7 days in culture medium (RPMI, L-glutamine, penicillin/streptomycin, 2-mercaptoethanol, HEPES, 10%FBS) with GM-CSF (10ng/mL) and of IL-4 (1μg/mL). CD11c+ DCs were purified by positive magnetic sorting (Miltenyi). Murine iNKT cell lines were derived from Vα14 TCR Tg mice as previously described (Chiba et al., 2009).

Preparation of A.a., H.c., C.n* and *C.a. *A.a.* was grown on PDA for 7 days, harvested in 5%DMSO/H_2O using a cell scraper, filtered, washed, re-suspended, autoclaved and sonicated as described for *A.f.* *H.c.* yeasts were cultured in Ham's F-12 to mid-log phase, harvested, counted and washed in LPS-free HBSS, then heat-killed at 60°C for 30 minutes. *C.n.* and *C.a.* yeasts were cultured in YPD until mid-log phase, washed in LPS-free PBS, counted and heat-killed at 75°C for 45 or 117°C for 30 minutes, respectively.

Sugar analysis of acid/base refluxed fungal pellet. Glycosyl composition of the biologically active insoluble pellet was determined by combined gas chromatography/mass spectrometry (GC/MS) of the per-*O*-trimethylsilyl (TMS) derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis (Merkle and Poppe, 1994; York et al., 1985). For glycosyl linkage analysis, the sample was enzymatically depolymerized, permethylated, further depolymerized, reduced, and acetylated; and the resultant partially methylated alditol acetates (PMAAs) analyzed by gas chromatography-mass spectrometry (GC-MS) (York et al., 1985).

Supplemental Figure 1

SUPPLEMENTARY FIG. 1



Supplemental Figure 2

SUPLLEMENTARTY FIG. 2