Supplementary Materials for

CX3CR1^{+} mononuclear phagocytes control immunity to intestinal fungi

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This PDF file includes:

Other Supplementary Materials for this manuscript includes the following:

Materials and Methods

Mice

Cx3cr1$^{GFP/GFP}$, C57BL/6J, *Cd11c-cre*, *Irf4*$^{fl/fl}$, *Cx3cr1*$^{tm3(DTR)Litt/J}$ (*Cx3cr1*^{DTR}), *Syk*$^{fl/fl}$, *Cx3cr1*$^{tm2.1(cre/ERT2)Jung}$/J (*Cx3cr1-cre/ERT2*$^{+/+}$), *Batf3*$^{-/-}$, Thy1.1, and OVA-specific CD4^{+} (OT-II) T-cell receptor-transgenic (*H-2*^{b}) mice were purchased from the Jackson Laboratories (Bar Harbor, ME). The *Cx3cr1*^{DTR} mice were subsequently bred to hemizygous *Cd11c-cre*$^{+/-}$ mice (expression of *Cd11c-cre* excises the floxed stop cassette, allowing for DTR expression in CD11c^{+} cells) to allow for selective depletion of CD11c^{+} CD11b^{+} CX3CR1^{+} MNPs upon administration of diphtheria toxin (DT). For depletion, mice generated through such breeding (*Cd11c-cre*$^{+/-}$ *Cx3cr1*^{DTR} mice) and the respective littermate controls (*Cd11c-cre*$^{-/-}$ *Cx3cr1*^{DTR}) were injected for three consecutive days with 100ng of DT i.p followed by maintenance injection every other day for the length of the experiments. Depleted mice were designated throughout the study as ΔCX3CR1 mice. *Irf4*$^{fl/fl}$ mice were bred to hemizygous *Cd11c-cre*$^{+/-}$ mice to allow for genetic ablation of CD11c^{+}CD11b^{+}CD103^{+} dendritic cells (*Cd11c-cre*$^{+/-}$ *Irf4*$^{fl/fl}$ mice, designated throughout the study as ΔIrf4 mice) and the generation of littermate controls (*Cd11c-cre*$^{-/-}$ *Irf4*$^{fl/fl}$ mice) (15). *Syk*$^{fl/fl}$ mice were bred to *Cx3cr1-cre/ERT2*$^{+/+}$ to allow for the selective depletion of Syk in CX3CR1 expressing cells upon administration of tamoxifen. For induction of Cre-ERT recombinase in *Cx3cr1-cre/ERT2*$^{+/-}$ mice, tamoxifen (TAM, Sigma) was dissolved in sunflower oil (Sigma) to a final concentration of 10 mg/ml, and 100μl/mouse were injected i.p. every second day. Control littermates were injected with 100μl sunflower oil (31). Mouse experiments were performed with at least four mice per group. Littermates were randomly assigned to experimental groups. Animals were used between 8 and 16 week of age. Males and females were used in approximately equal ratios. All animals were housed under specific pathogen-free conditions unless otherwise described at Weill Cornell Medicine and experiments were performed after prior approval by the Institutional Animal Care and Use Committee of Weill Cornell Medicine.

Fungal strains and generation of *Candida albicans* strain expressing OVA-II

Candida albicans (SC5314) and *Candida tropicalis* (ATCC 750) were obtained from the American Type Culture Collection (Manassas, VA). *C. albicans* expressing red fluorescent protein (*C.a*-RFP) was generated as described (32). *Saccharomyces cerevisiae* (ATCC® MYA-796™) and all *Candida* strains were cultured in aerobic conditions on Sabouraud Dextrose Broth (SDB; EMD Chemicals) at 37ºC. *Aspergillus amstelodami* (ATCC 46362) and *Aspergillus versicolor* (ATCC 52173) were cultured on SDB at 30ºC. *Wallemia sebi* (FRR 1471, ATCC 42964) was grown on Sabouraud Dextrose Agar (SDA; EMD Chemicals) at RT. *Malassezia restricta* (ATCC MYA-4611) was grown on Sabouraud Dextrose Agar (SDA; EMD Chemicals) overlaid with olive oil at 37ºC. For the generation of *Candida albicans* strain expressing GFP-OVA Class II Epitope fusion (C.a-OVA) standard molecular biology procedures were used. Briefly, plasmids were amplified in *Escherichia coli* DH5α strain growing in LB medium at 37ºC supplemented with ampicillin (100 mg/L). In order to generate strains expressing a GFP-OVA Class II Epitope fusion, we first constructed the OVA Class II Epitope (ISQAVHAAHAEINEAGR) using the primers o-OVAII-up (CCGGGGATCCGAATCTTTGAAAATTTCTCAAGCTGT TCACGCTGCTCACGCTGAAATTAACGAAGCTGGTAGAGAAGTTGTTGGTGAT CTGGCC) and o-OVAII-low (GCCAGATCTACCAACAACTTCTCTACCAGCTTCG

TTAATTTCAGCGTGAGCAGCGTGAACAGCTTGAGAAATTTTCAAAGATTCGG ATCCCCGG). PCR conditions: 94ºC for 1 minute, annealed at 55ºC for 5 min and then cooled to 4ºC. The resulting small dsDNA was then digested with *Bam*HI and *Bgl*II and ligated to the yeast YEP352 episomal plasmid in the *Bam*HI site. Plasmids carrying the class II epitope were selected by sequencing of the recombinant plasmids. The sequence of a *C. albicans* adapted GFP version (33) was amplified using the primers GFPMo-up (GGCCGGATCCGCGGCCGCATGAGTAAAGGAGAAGAACTTTTC) and GFPMo-lo (GGCCAGATCTTTTGTATAGTTCATCCATGCCATGTGT) and inserted at the *Bam*HI site of the previous construct to generate a C-terminal in frame GFP fusion. This construct (Fig. S11) was transferred to a *Sal*I-*Bgl*II treated *C. albicans* vector pNIM1, a doxycycline inducible plasmid (34), by amplification using the primers JES13 (ATATAAATACTCGAGAAAGATGAGTAAGGGAGAAGAACTTTTC) and JES15 (ATTTAGATCTTTACATGATGCGGCCCTCCTGCAG) which introduced *Xho*I (*Sal*I - compatible) and *Bgl*II sites. Finally, to make the system repressible (instead of inducible), an *Ava*I-*Kpn*I piece of DNA from plasmid pNIM1R (kindly provided by Leah Cowen) replaced the *Ava*I-*Kpn*I portion of the above construction (32). The final plasmid, pNIM1R-GFP-OVAII was integrated at the *C. albicans* genome by digestion with *Sac*II and *Kpn*I, directing the integration at the *ADH1* region of wild type CAF2 strain (35). Strains were transformed by electroporation and transformants were selected as nourseothricine resistant clones on YPD medium supplemented with nourseothricine (100 mg/L). GFP and OVA Class II epitope expression were confirmed by flow cytometry and in *in vivo* assays as described below.

Imaging of in-vivo and ex-vivo fungal intake by $CX3CR1^{+}$ mononuclear phagocytes

For the imaging of in vivo uptake of *C. albicans, Cx3cr1*$^{GFP/GFP}$ were bred to C57BL/6J mice and the resulting heterozygous mice were fed for 3 consecutive days with $5 \cdot 10^7$ cfu/mouse *C.a*-RFP. Mice were sacrificed at day 4, colon were excised and gently flushed with cold PBS to remove feces. Colons were cut in segments, flushed twice with PBS, fixed and stained as described below. For the imaging of the ex-vivo uptake of fungi, colons from *Cx3cr1*$^{GFP/+}$ mice were excised and gently flushed with complete 37ºC RPMI media to remove the feces (36). Colon segments were closed with surgical threads and fungi ($2 \cdot 10^7$ /ml in complete RPMI) were injected. Sections were incubated for 2h at 37°C. Sections were washed, fixed and blocked with 1ml PBS, 2% BSA, followed by Hoechst or DAPI staining. Sections were finally washed 3x with water and mounted on glass chamber with Invitrogen Prolong Gold Antifade (ThermoFisher). Images were acquired on a Leica TCS SP confocal microscope with a Leica PL APO 100x/1.4 oil immersion lens.

Genotyping and serological analysis

Crohn's disease subjects were recruited by the IBD Center at Cedars-Sinai Medical Center following informed consent and IRB approval. DNA samples from human peripheral blood or B-lymphoblastoid cell line specimens were processed using an Illumina Infinium HumanExome+ BeadChip at Cedars-Sinai Medical Center and underwent quality control as previously described (37). Three missense SNPs passing quality control spanned the CX3CR1 gene (rs3732378, rs3732379, rs41535248) and a total of 503 Jewish CD subjects with complete data in genetic and serological markers were included in the genetic analysis. Serological evaluation was performed by ELISA as described below.

Detection of anti-fungal and anti-bacterial antibodies in the mouse and human serum

The level of antibodies in the mouse or human serum that react with bacterial or fungal antigens was determined by a sandwich enzyme-linked immunosorbent assay (ELISA). Briefly, to obtain bacterial or fungal lysates for ELISA the overnight fungal or bacterial colonies were prepared through freeze and thawing disruption followed by 5 pulses of sonication. Such microbial lysates, *Saccharomyces cerevisiae* mannan (Sigma) or bacterial flagellin (Cbir-1) were used as the coating antigen in ELISA assay. ELISA detection of IgG was carried out as previously described (38). Samples were read at 405 nm on a microtiter plate reader (Menlo Park, CA).

Induction of DSS colitis and fungal targeting with fluconazole

Cx3cr1^{DTR} or littermate control mice were injected with DT for three consecutive days, prior to the induction of colitis with 3% (w/v) Dextran sulphate sodium (DSS, MP *Biomedicals*, *LLC*, Aurora, OH) in the drinking water. DT was then administered every second day and DSS water was removed at day 7. To target intestinal fungi, mice were given Fluconazole (0.5 mg/ml, Sigma) in the drinking water for the duration of the DSS treatment, starting 4 days prior the first DSS dose. In some experiments, mice were orally supplemented with *Candida tropicalis* or *Candida albicans* ($5 \cdot 10^7$ yeast/mouse/dose) every other day starting 2 days prior the DSS treatment. Control groups were orally gavaged with PBS at the same time points. Body weight and the presence of occult blood were assessed daily. Due to the increased intestinal disease susceptibility of ΔCX3CR1 mice, all mice were sacrificed at days 7 to 10 from the first DSS dose. Colons, ileum and mLNs were removed for analysis. Distal colonic samples were fixed in 5% formalin and paraffin embedded. Slides were stained with hematoxylin and eosin (H&E) and imaged by light microscopy.

Colonization of germ-free mice

For microbiota transfer experiments, feces were collected and pooled from 5 ΔCX3CR1 mice and from 5 control littermates after 1 week of treatment with DT. The pellets were resuspended in PBS and gavaged to 6 week-old germ-free mice. 16S rRNA qPCR of fecal content was used to confirm colonization. 2.5% DSS was administered in the drinking water and mice were sacrificed at day 7 as described above.

Assessment of *Candida* induced immune response and adoptive transfer of $CD4^+$ $Thy1.1^+$ OT-II cells

Cefoperazone (0.4 mg/ml; Sigma-Aldrich, St. Louis, MO) was provided to mice ad libitum in drinking water for the whole length of the experiments. Mice were fed with $5 \cdot 10^7$ *C. albicans* cells at day 1, 4 and 7 after the start of the cefoperazone water. Mice were sacrificed at day 10 and sampled as described below.

For the analysis of Candida specific T cell responses, spleens and mesenteric lymph nodes of $CD4^+$ $Thy1.1^+$ OT-II donor mice were removed by dissection. Tissues were mashed onto a nylon screen, and the cells obtained were pooled, washed twice in Hanks' balanced salt solution (HBSS; Sigma-Aldrich), and resuspended at $1 \cdot 10^8$ cells/ml. Transgenic $CD4^+$ $Thy1.1^+$ OT-II T cells were isolated by negative selection, using MagniSort™ Mouse $CD4^+$ T cell Enrichment Kit (eBioscience), according to the manufacturer's protocol. The purity of the $CD4^+$ cell population in the enriched fraction was >95%, as determined by flow

cytometry analysis. $CD4^+$ isolated T cells were pooled and stained with carboxy-fluorescein diacetate succinimidyl ester (*CFDA*-SE; 7.5µM Invitrogen) for 10 min at 37°C. $5 \cdot 10^6$ of CFSE-labeled T cells were transferred into recipient mice by retro-orbital injection. One day later, mice were fed with C.a-OVA as described above. The inoculum was provided at day 1, 4 and 7 to avoid clonal expansion of *Candida* and loss of the OVA peptide antigen. Mice were terminated after 10 days. Successful adoptive transfer was confirmed by analysis of the $CD4^+$ $Thy1.1^+$ population in the spleen, MLNs, colon and small intestines.

Isolation of intestinal mucosa, small and large intestine lamina propria cells

Intestinal LP cells were isolated as previously described (39) with some modifications. Briefly, colons were isolated, opened longitudinally, washed of fecal contents and then cut into 1 cm pieces. Intestinal pieces were transferred into HBSS medium (Sigma), supplemented with 5% fetal bovine serum (FBS) and 2 mM EDTA, and were shaken for 8 min at 37°C. The suspensions were filtered through a mesh and the filtrate containing epithelial cells fraction were washed twice with PBS and used further for RNA and DNA isolation. The remaining tissue was washed, cut in small pieces and subsequently incubated in digestion medium consisting of RPMI 1640, 5% FBS, 0.5 mg/ml collagenase type VIII (Sigma), 5 U/ml DNase (Roche Diagnostics), 100 IU/ml penicillin and 100 µg/ml streptomycin for 25 min at 37°C by gentle shaking. The cell suspensions were filtered through a mesh, overlaid on percoll (39) or used directly for immunophenotyping of LP cells.

Antibodies and flow cytometry

Cell suspensions were prepared as described above, blocked with CD16/CD32 (Mouse BD Fc Block™, 2.4G2, BD Biosciences) and stained with antibodies against CD4 (GK1.5, eBioscience), CD45 (30-F11, Tonbo), CD90.1 (OX-7, Biolegend), CD11b (M1/70, Tonbo), CD11c (N418, Tonbo), CD64 (X54-5/7.1 FC, Biolegend), CD103 (2E7, eBioscience), MHC-II (I-A/I-E, M5/114.15.2, eBioscience), NK1.1 (eBioscience, PK136), Ly-6G (RB6-8C5, Tonbo), CX3CR1 (SA011F11, Biolegend), CD40 (HM40, eBioscience), CD86 (Gl-1, Biolegend), Dectin-1 (2A11, Bio-Rad), Dectin-2 (KVa7-6E7, Miltenyi). For intracellular staining of transcription factors, cells were stained with surface markers, fixed permeabilized and stained with FoxP3 (FJK-16s, eBioscience) and RORγt (B2D, eBioscience). For intracellular cytokine and Syk detection, cells were incubated with 50 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich), 500 ng/ml ionomycin (Sigma-Aldrich) and 10 µg/ml Brefeldin A (BFA; Sigma-Aldrich) in complete RPMI media at 37° C for 6 h. After surface staining with CD45, CD4, Thy1.1 cells were fixed, permeabilized and staining was performed using PE-labeled anti-IL-17 mAb (eBio17B7; eBiosciences) or PE-labelled anti-Syk mAb (5F5; Biolegend) according to the manufacturer's instructions. Flow cytometry was performed using a LSRFortessa (BD Biosciences) and data were analyzed with FlowJo software (TreeStar Inc.).

DNA isolation, fungal and bacterial rDNA gene quantitative analysis

Fecal pellets were collected as indicated in the text, DNA for fungal and bacterial sequencing and validation RT-qPCR was isolated from 2-3 fecal pellets following lyticase treatment, bead beating, and processing using QIAmp DNA mini kit (Qiagen) as in(40).

Microbiome sequencing analysis, Illumina library generation and sequencing

Mouse fungal and bacterial microbiomes were sequenced using the Illumina MiSeq platforms. Fungal ITS1-2 regions and bacterial 16S regions were amplified by PCR using primers modified to include sample barcodes and sequencing adaptors:

Fungal primers:

ITS1F CTTGGTCATTTAGAGGAAGTAA
ITS2R GCTGCGTTCTTCATCGATGC

Bacterial primers:

16S-27F AGAGTTTGATCMTGGCTCAG
16S-BSR357R CTGCTGCCTYCCGTA

Forward overhang:

5’ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus-specific sequence]

Reverse overhang:

5’ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[locus-specific sequence]

ITS1 amplicons were generated with 35 cycles, whereas 16S amplicons were generated with 25 cycles using Invitrogen AccuPrime PCR reagents (Carlsbad, CA). Amplicons were then used in the second PCR reaction, using Illumina Nextera XT v2 (San Diego, CA) barcoded primers to uniquely index each sample and 2x300 paired end sequencing was performed on the Illumina MiSeq (Illumina, CA). DNA was amplified using the following PCR protocol: Initial denaturation at 94^{o}C for 10 min, followed by 40 cycles of denaturation at 94^{o}C for 30 s, annealing at 55^{o}C for 30 s, and elongation at 72^{o}C for 2 min, followed by an elongation step at 72^{o}C for 30 min. All libraries were subjected to quality control using qPCR, DNA 1000 Bioanalyzer (Agilent), and Qubit (Life Technologies) to validate and quantitate library construction prior to preparing a Paired End flow cell. The sequencing data are deposited in NCBI Sequence Read Archive (SRA, http://www.ncbi.nlm.nih.gov/Traces/sra, run number SRP124782, SRP124783, SRP124736, SRP124742).

Data Analysis

Raw FASTQ ITS1 sequencing data were filtered to enrich for high quality reads, removing the adapter sequence by cutadapt v1.4.1 or any reads that do not contain the proximal primer sequence (40). Sequence reads were then quality-trimmed by truncating reads not having an average quality score of 20 (Q20) over a 3 base pair sliding window and removing reads shorter than 100 bp (40). These high quality reads were then aligned to Targeted Host Fungi (THF) ITS1 database, using BLAST v2.2.22 and the pick_otus.py pipeline in the QIIME v1.6 wrapper with an identity percentage ≥97% for operational taxonomic unit (OTU) picking (41). The alignment results were then tabulated across all reads, using the accession identifier of the ITS reference sequences as surrogate OTUs and using a Perl script (40). For Illumina bacterial analysis, because of the abundance of

Illumina reads and higher overall sequence quality of the reads, we used the QIIME package with minimal customization (42). Shannon diversity index (43) (H) was calculated at the OTUs levels as (1)

$$H^I = -\sum_{i=1}^{S} p_i \ln(p_i) \quad (1),$$

where p_i is the proportional abundance of OTUs *i.*

Simpson Diversity Index (1-D)(44) was calculated as (2)

$$1 - D = 1 - \frac{\sum_{i=1}^{R} n_i(n_i-1)}{N(N-1)} \quad (2)$$

Where N= total number of individuals of all species, n_i = total number of individuals for each species *i.*

For the diversity analysis at the Phylum level, Shannon and Simpson diversity at the OTU level were calculated within the two major gut associated bacterial (Firmicutes and Bacteroidetes) and fungal (Ascomycota and Basidiomycota) phyla.

Real Time qPCR

Total fecal DNA isolated was isolated as described above. Real-time RT-PCR analyses were done on the Applied Biosystems 7500 Fast Real-Time PCR System with the SYBR Green PCR kit as instructed by the manufacturer (Applied Biosystems). Samples were analyzed for gene expression using the following primers:

Gene	*Forward*	*Reverse*	*Ref*
Eubacteria	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC	(45)
SFB	AGGAGGAGTCTGCGGCACATTAGC	TCCCCACTGCTGCCTCCCGTAG	(46)
Fungi (18S)	ATT GGA GGG CAA GTC TGG TG	CCG ATC CCT AGT CGG CAT AG	(47)
Candida spp.	CCTGTTTGAGCGTCGTTT	TCCTCCGCTTATTGATAT	(48)
C. tropicalis	TTTGGTGGCGGGAGCAATCCT	CGATGCGAGAACCAAGAGATCCGT	(3)
ITS5	GGAAGTAAAAGTCGTAACAAGG		(49)
Ascomycota-specific-R	ITS5	CGTTACTRRGGCAATCCCTGTTG3	(49)
Basidiomycota-specific-R	ITS5	GCRCGGAARACGCTTCTC3	(49)

Gene expression profiling by RNA-seq and bioinformatics analyses

Live lineage negative (CD3, CD19, ab-TCR, SiglecF, Ly6G) $CD45^+MHCII^+CD11b^+$ $CD11c^+$ $CX3CR1^+$ and live lineage negative $CD45^+MHCII^+CD11b^+$ $CD11c^+$ $CD103^+$ cells were sorted from the colon of *Cx3cr1*$^{GFP/+}$ mice (Fig. S1A). cDNAs were prepared using the SMARTer Ultra Low Input RNA Kit for Sequencing version 3 following the user manual (Clontech Laboratories). Sequencing libraries were prepared using the Nextera XT DNA Library Prep Kit (Illumina). Cluster generation and 75–base pair single-end dual-indexed sequencing was performed on the Illumina NextSeq 500 system. The raw reads of RNA-seq were aligned to the mm10 genome using TopHat v1.0; packages Rsamtools v1.24.0, GenomicFeatures (50). Genes where annotated to their Entrezid (package org.Mm.eg.db) leaving 24'451 total genes. Genes whose count per millions (CPM) was below 1 were removed from further processing, leaving 23707 total genes (51), Raw data are deposited in *GEO* (http: //www.ncbi.nlm.nih.gov/geo/) under accession number GSE106594. The counts were analyzed for differential expression using EdgeR. For the analysis of genes involved in fungal recognition and antigen presentation expressed genes

belonging to the following GO terms were selected: Fungal recognition: GO:0009620, GO:0071226, GO:0016046, GO:0002238, GO:0009610, GO:0001878; Antigen Presentation: GO:0042742.

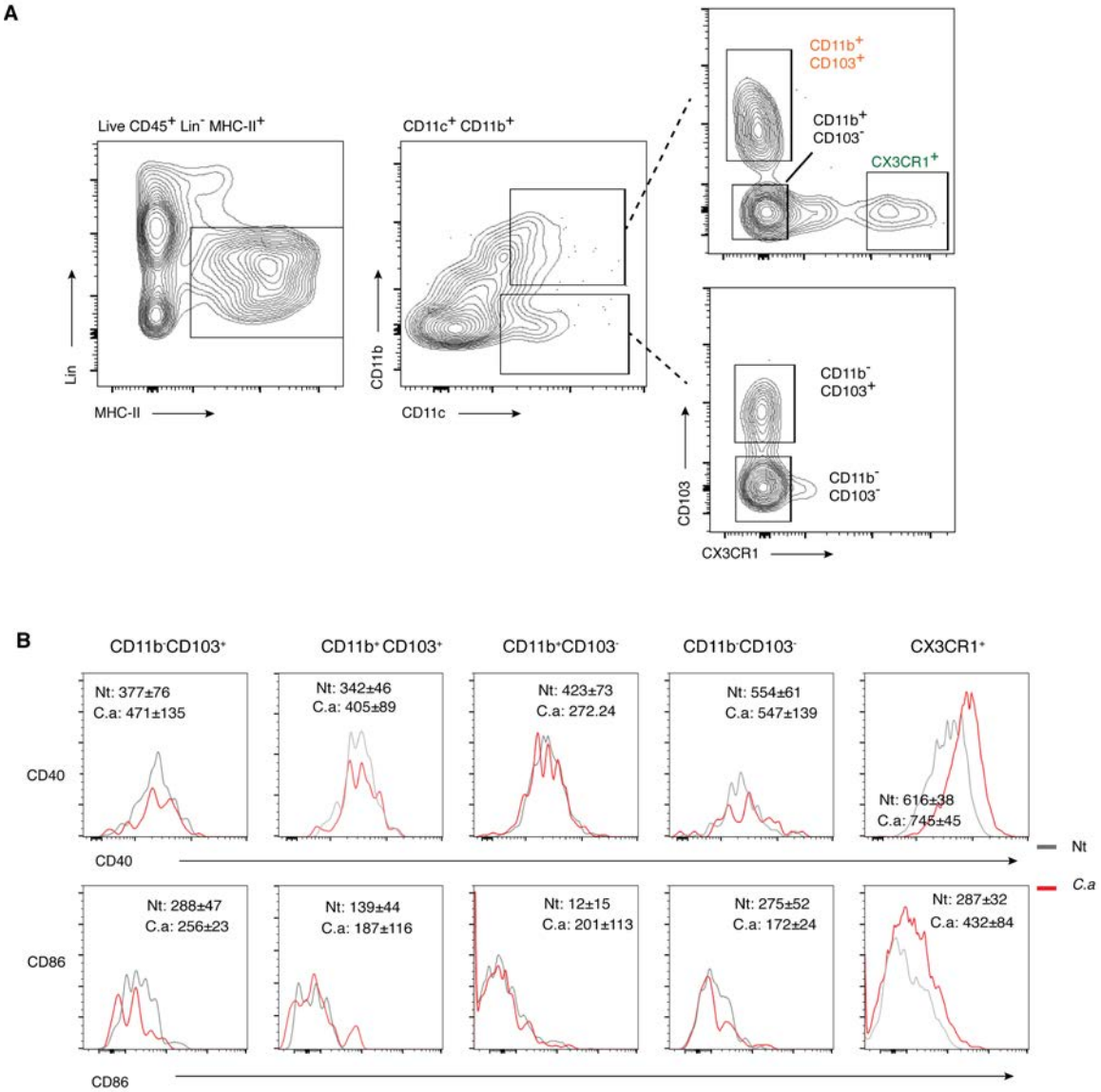

Fig. S1. Cell sorting strategy and costimulatory molecules expression by intestinal phagocytes upon *C. albicans* colonization.

(A) LP cells were isolated as described in the method section. Cell sorting strategy for purifying phagocytic populations from the colon of *Cx3cr1*$^{GFP/+}$ mice. **(B)** For the quantification of CD40 and CD86 expression among phagocytic populations following *C. albicans* colonization, cells were first gated on singlet, live and lineage negative (CD3$^-$, CD19$^-$, TCRβ$^-$, Ly6C$^-$, NK1.1$^-$) CD11c$^+$ populations, and subdivided into CD11b$^+$ CD103$^+$; CD11b$^+$ CD103$^-$; CD11b$^+$ CX3CR1$^+$; CD11b$^-$ CD103$^+$ and CD11b$^-$ CD103$^-$ subsets.

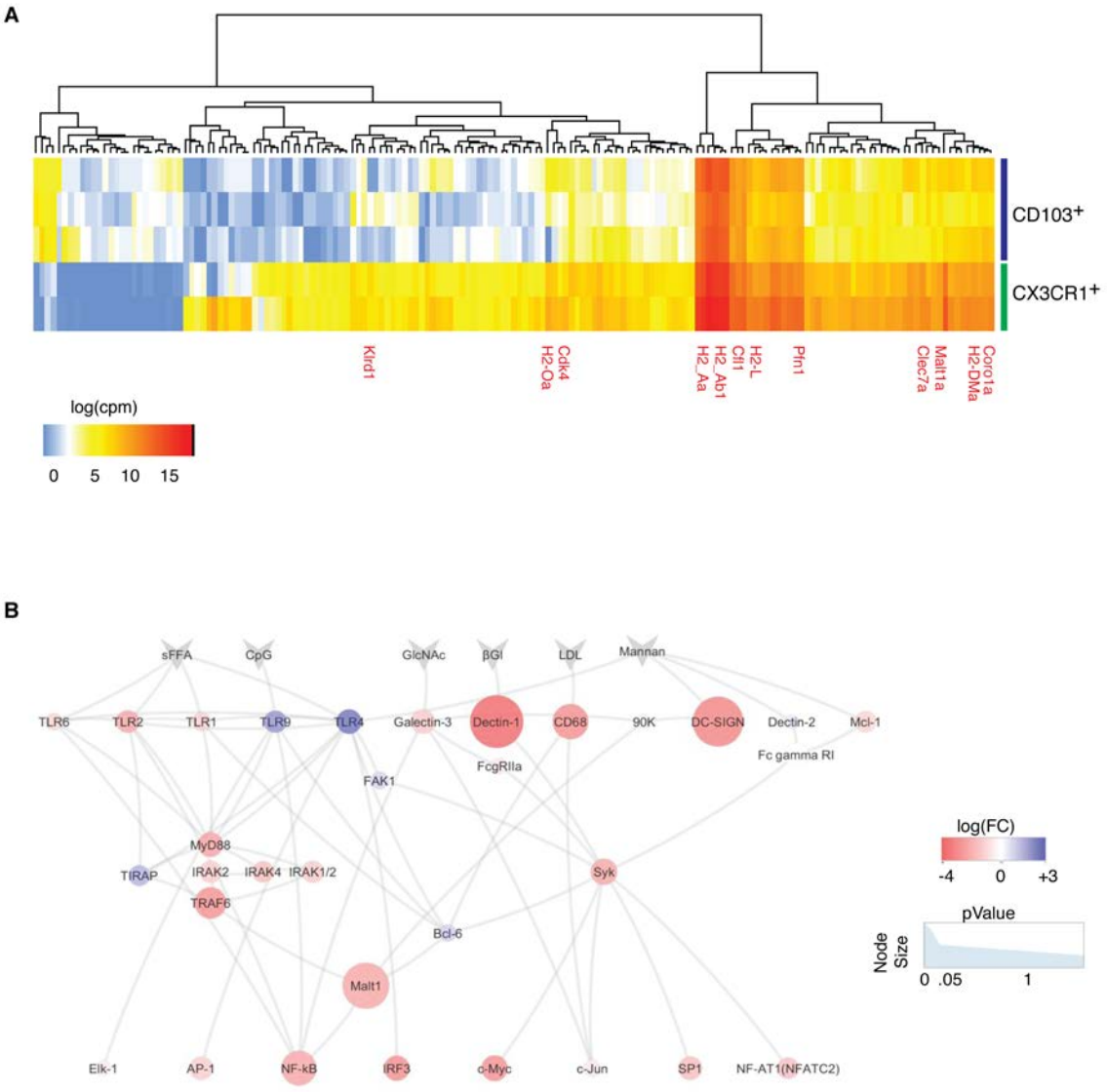

Fig. S2. RNA sequencing analysis of sorted CD11b$^+$ CD103$^+$ DCs and CX3CR1$^+$ MNPs.

(A) Cells were sorted as in Fig S1. Genes were clustered on the y axis based on Euclidean measures. Blue/red unsupervised clustering/heatmap on the left indicate log count per millions [log(cpm)] of the expressed genes in sorted CD11b$^+$ CD103$^+$ cells and CD11b$^+$ CX3CR1$^+$ cells. **(B)** Network of expressed genes involved in fungal recognition was generated using Metacore (Thomson Reuter) Network was visualized with Cytoscape.

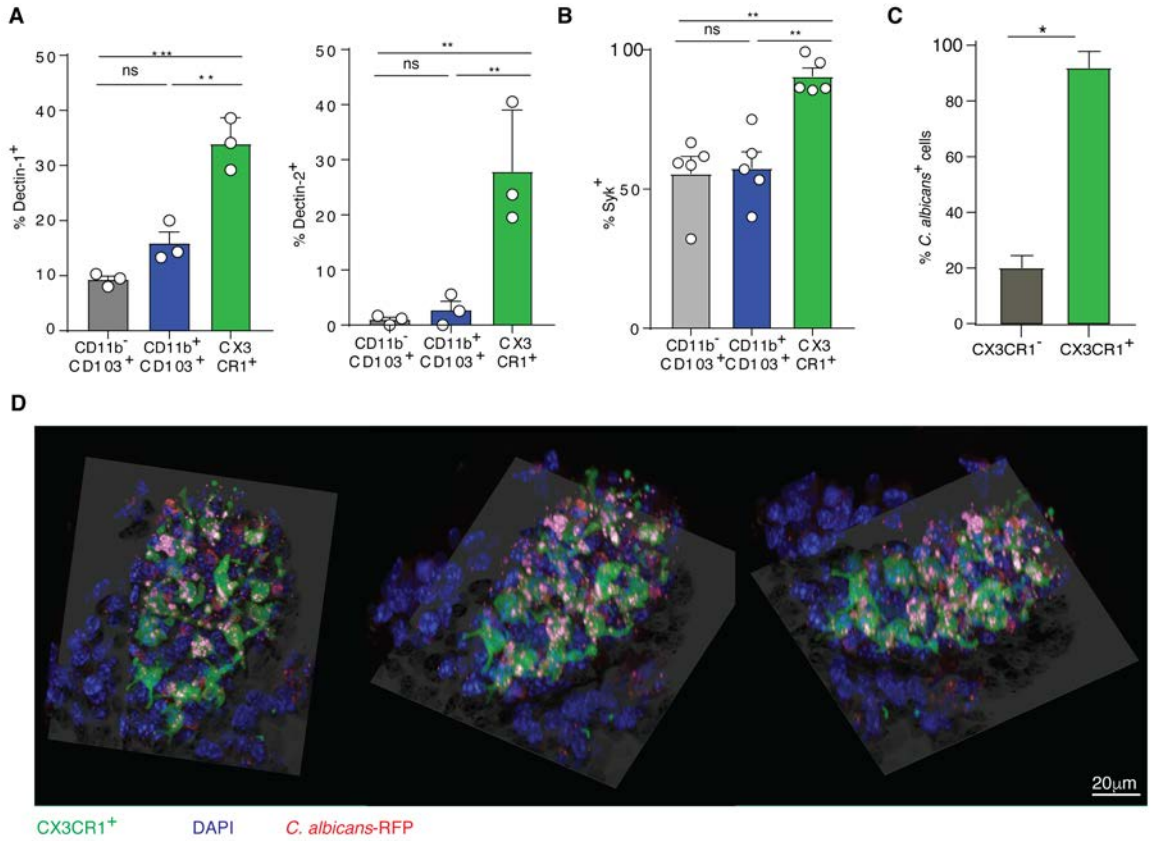

Fig. S3. CLR expression by intestinal phagocytes and *C. albicans* uptake by CX3CR1$^+$ MNPs.

Quantification of dectin-1, dectin-2 **(A)** and Syk **(B)** expression by flow cytometry among CD11b$^-$ CD103$^+$ DCs; CD11b$^+$ CD103$^+$ DCs and CD11b$^+$ CX3CR1$^+$ MNPs in colons of C57BL6 mice**.** Quantification **(C)** and 3D rendering **(D)** of confocal stack showing intake of *C. albicans*-RFP by CX3CR1$^+$ and CX3CR1$^-$ cells in the intestines of *Cx3cr1*$^{GFP/+}$mice**.**

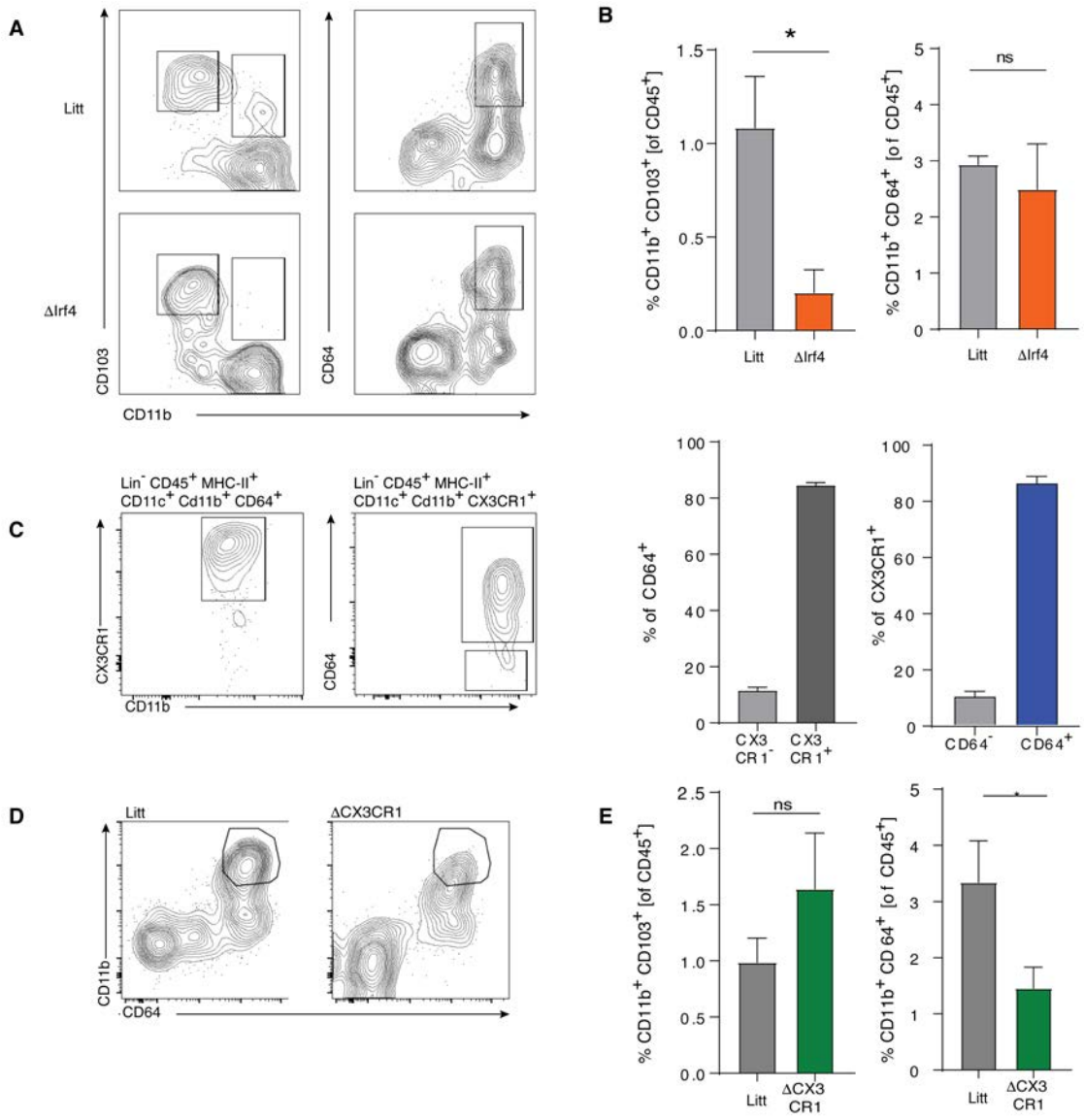

Fig. S4. Depletion of gut resident phagocytes.

(A) Representative flow cytometry plot of the colonic, Lin$^-$CD45$^+$MHC-II$^+$CD11c$^+$ lymphocytes in ΔIrf4 (*Cd11c-cre*$^{+/-}$ *Irf4*$^{fl/fl}$ mice) mice or wild type (*Cd11c-cre*$^{-/-}$ *Irf4*$^{fl/fl}$ mice) littermates (Litt). **(B)** Quantification of the CD11b$^+$ CD103$^+$ and CD11b$^+$ CD64$^+$ subsets among and CD45$^+$ cells**. (C)** Expression of CX3CR1 among, Lin$^-$ CD45$^+$MHC-II$^+$ CD11c$^+$ CD11b$^+$ CD64$^+$ cells and of CD64 among Lin$^-$ CD45$^+$MHC-II$^+$ CD11c$^+$ CD11b$^+$ CX3CR1$^+$ cells. CD64 is expressed on CX3CR1$^+$ MNPs and is used to quantify their depletion in (D) and (E). **(D)** Representative flow cytometry plot of colonic, Lin$^-$ CD45$^+$ MHC-II$^+$ CD11c$^+$ cells in ΔCX3CR1 mice (*Cd11c-cre*$^{+/-}$ *Cx3cr1*^{DTR} + DT) or wild type (*Cd11c-cre*$^{-/-}$ *Cx3cr1*^{DTR} + DT) littermates (Litt). **(E)** Quantification of the CD11b$^+$ CD64$^+$ and CD11b$^+$ CD103$^+$ subsets among CD45$^+$ cells. Data expressed as mean ± SEM of individual mice, representative of three to five independent experiments.

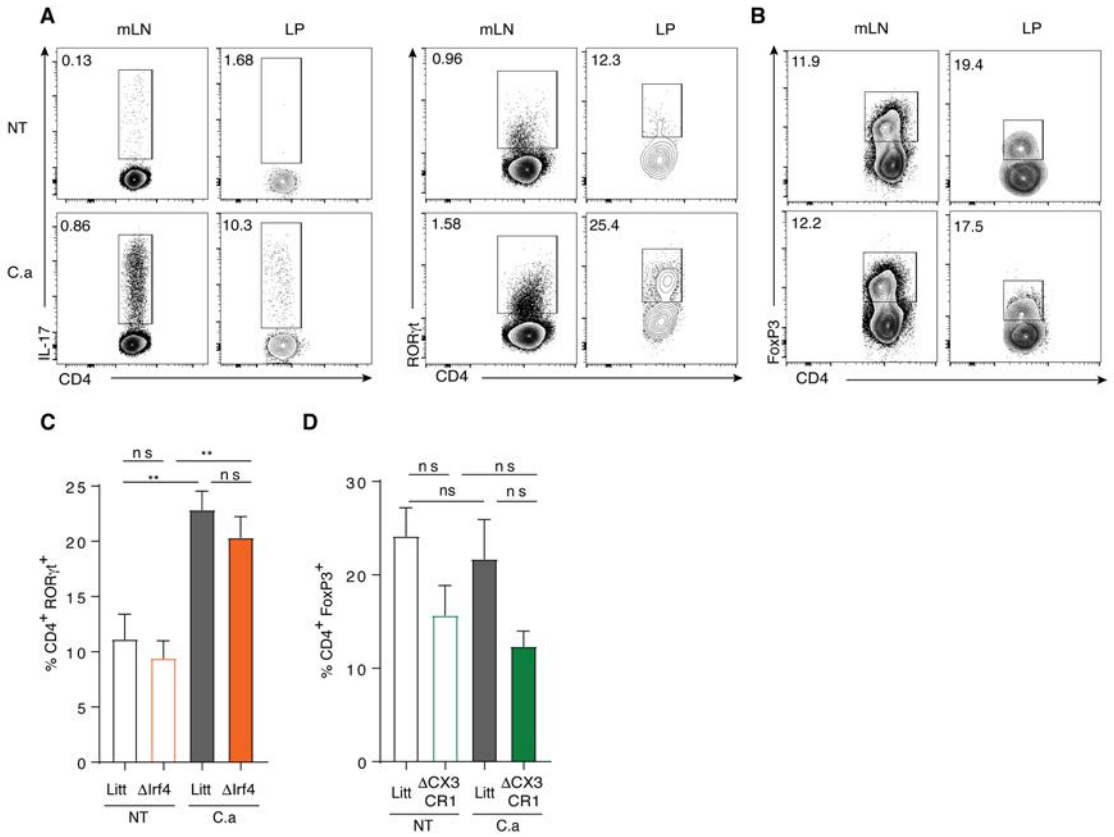

Fig. S5. Quantification of Th17 and Treg cells in the intestine and mesenteric lymph nodes (mLN) following *C. albicans* colonization.

Mice were fed with $5 \cdot 10^7$ CFU *C. albicans* (C.a) every 3 days for 10 days. **(A)** Representative plot of IL-17 and RORγt expression among $CD4^+$ T cells in the mLN (left) and colonic lamina propria (right) following *C. albicans* administration**. (B)** Representative plot of FoxP3 expression among $CD4^+$ T cells in the mLN (left) and colonic lamina propria (right) following *C. albicans* administration**.** (**C)** Expression of RORγt in the colon of ΔIrf4 mice or control littermates (Litt). **(D)** Quantification of the $FoxP3^+$ Treg cells among the $CD4^+$ T cells in the colon of ΔCX3CR1 mice or wild type littermates. Data expressed as mean ± SEM of individual mice, representative of three to five independent experiments.

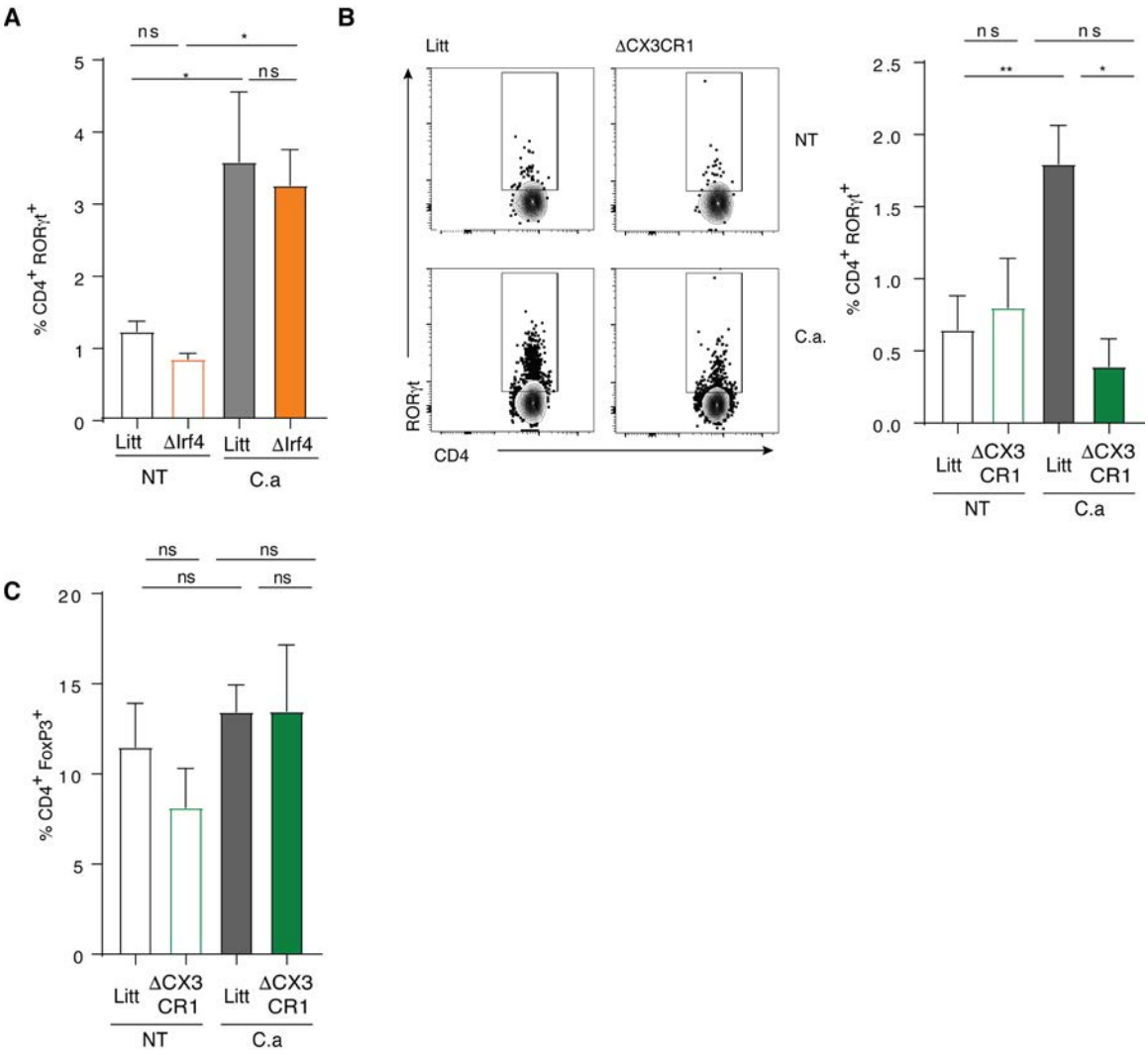

Fig. S6. Quantification of Th17 and Treg cells in the mesenteric LN (mLN) following *C. albicans* colonization.

(A) Quantification of RORγt$^+$ Th17 among the CD4$^+$ T cells in ΔIrf4 mice or wild type littermates. Representative flow cytometry plot and quantification of RORγt$^+$ Th17 **(B)** and FoxP3$^+$ Treg cells among the CD4$^+$ T cells in ΔCX3CR1 mice or wild type littermates **(C)**. Data expressed as mean ± SEM of individual mice, representative of three independent experiments. **P*<0.05, ***P*<0.01, ****P*<0.001, one-way ANOVA.

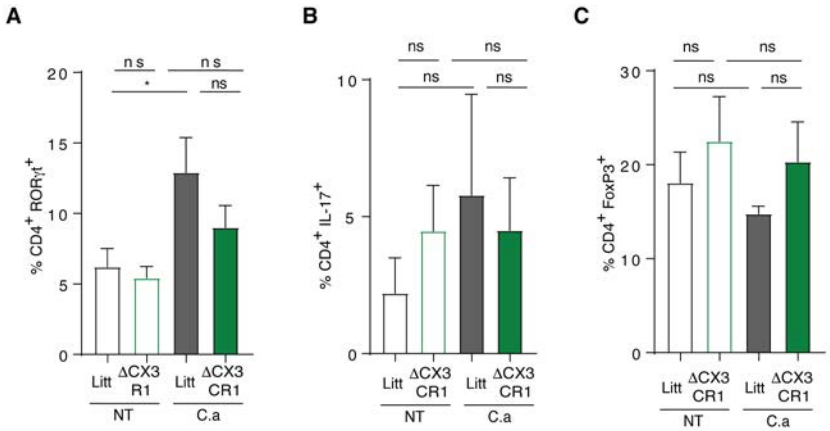

Fig. S7. Quantification of Th17 and Treg cells in the small intestine following *C. albicans* colonization.

Quantification of RORγt$^+$ Th17 **(A)**, expression of IL-17 **(B)** by CD4$^+$ T cells, FoxP3$^+$ Treg cells **(C)** and in the small intestine of ΔCX3CR1 mice or wild type littermates. Data expressed as mean ± SEM of individual mice, representative of three independent experiments.

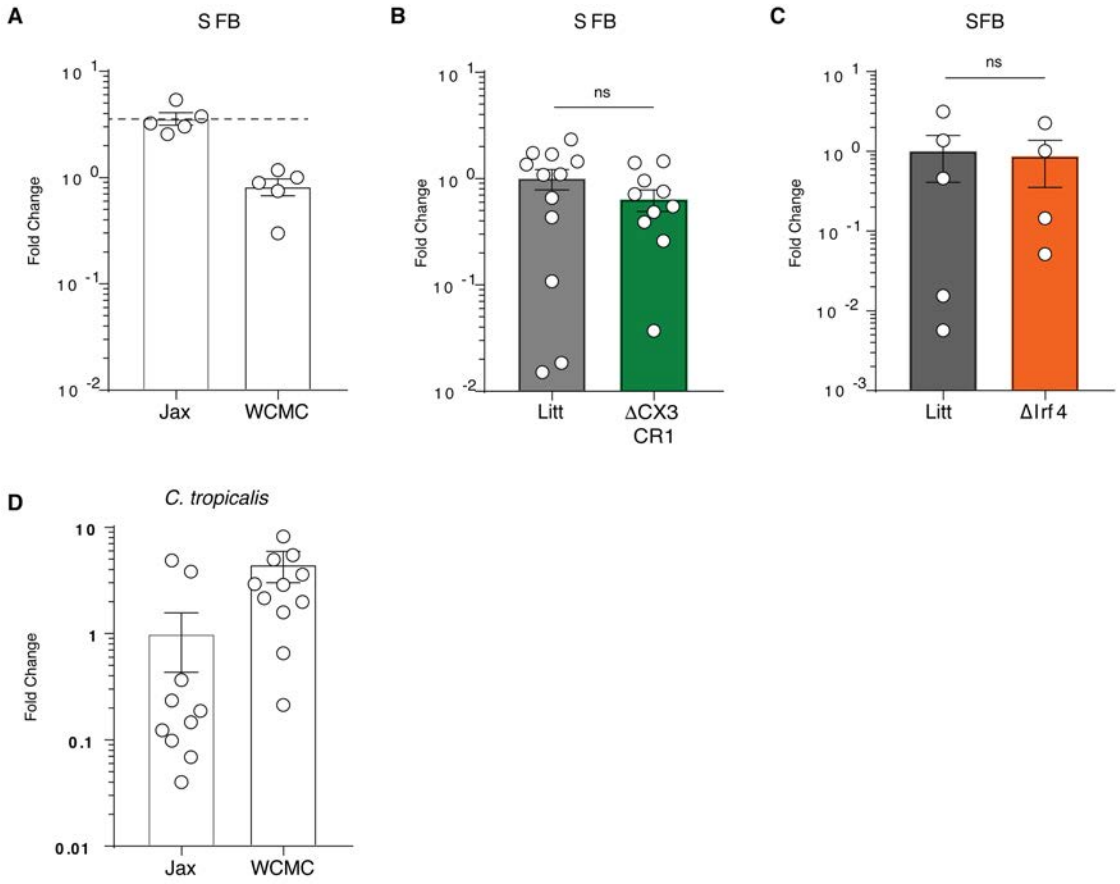

Fig. S8. RT-qPCR assessment of segmented filamentous bacteria (SFB) and *Candida tropicalis*.

(A) Relative abundance of SFB in C57BL6 mice purchased from Jaxon (Jax, considered SFB negative) relative to CD11c-cre$^-$ mice on a C57BL6 background housed in the Weill Cornell Medical Center (WCMC) facility after normalization to total bacteria (16S). **(B)** Abundance of SFB in ΔCX3CR1 mice relative to CD11c-cre$^-$ littermates after normalization to total bacteria (16S). **(C)** Abundance of SFB in ΔIrf4 mice relative to CD11c-cre$^-$ littermates after normalization to total bacteria (16S). **(D)** Relative abundance of *C. tropicalis* in C57BL6 mice purchased from Jaxon (Jax, considered SFB negative) relative to mice on a C57BL6 background housed in WCMC facility after normalization to total fungi (18S). Data expressed as mean ± SEM, dot represent individual mice. ns: not significant (Mann Whitney Test).

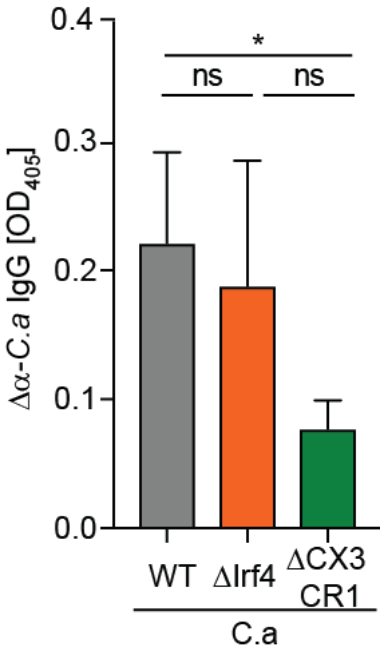

Fig. S9. RT-Induction of systemic IgG responses in ΔIrf4, ΔCX3CR1 and control littermates (WT) fed with *C. albicans.*

Mice were fed with 5.10^7 CFU *C. albicans* (C.a) every 3 days for 10 days and blood was collected at day 10. Dots represent individual mice. *P<0.05, **P<0.01, ***P<0.001, one-way ANOVA.

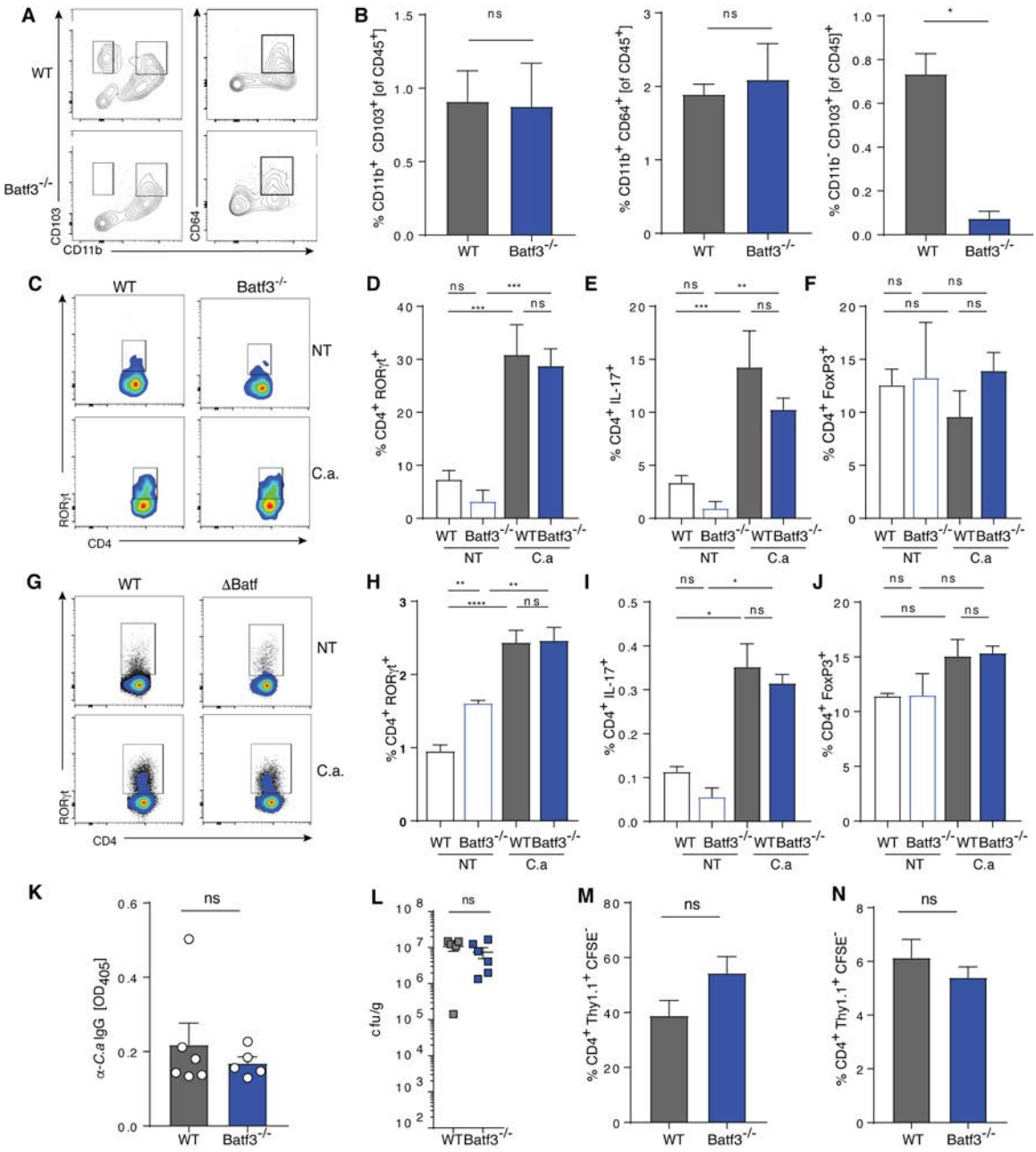

Fig. S10. Role of CD11b^{-} CD103^{+} DCs in the induction of antifungal responses.

(**A, B**) CD11b^{+}CD103^{+}, CD11b^{+}CD64^{+}, CD11b^{-}CD103^{+} subsets among CD45^{+} cells in Batf3$^{-/-}$ mice or wild type (WT) mice. **(C, D)** RORγt^{+} Th17 in the colon. Colonic Th17 (**E**) and FoxP3^{+} Treg cells (**F**). CD4^{+} RORγt^{+} **(G, H, I),** CD4^{+} IL-17^{+} **(H, I)** Th17 cells and FoxP3^{+} Treg cells (**J**) in the mLNs. (**K**) Systemic serum IgG responses and (**L**) *Candida* load in the feces in mice fed with *C. albicans*. Proliferating CFSE^{-} CD4^{+} Thy1.1^{+} OT-II cells in colon (**M**) and mLNs (**N**). Data expressed as mean ± SEM of individual mice, representative of two independent experiments. **P*<0.05, ***P*<0.01, ****P*<0.001, Mann Whitney (B, K-N) and one-way ANOVA (D-F,H-J).

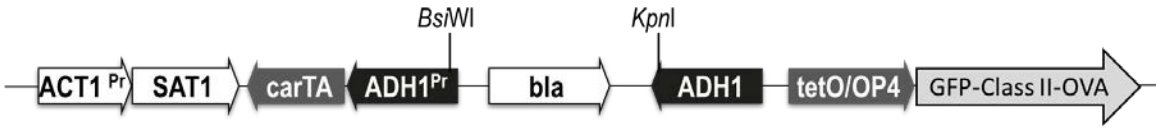

Fig. S11. Construct for the generation of *Candida albicans* strains expressing GFP-OVA Class II epitope fusion (C.a-OVA).
The *C. albicans*-adapted reverse trans activator activates the expression of GFP/ OVA Class II fusions via the tetO/OP4 promoter. Integration takes place at the *ADH1* region of the genome via digestion with *Bsi*WI and *Kpn*I and selection on nursothricine (*SAT1*) plates.

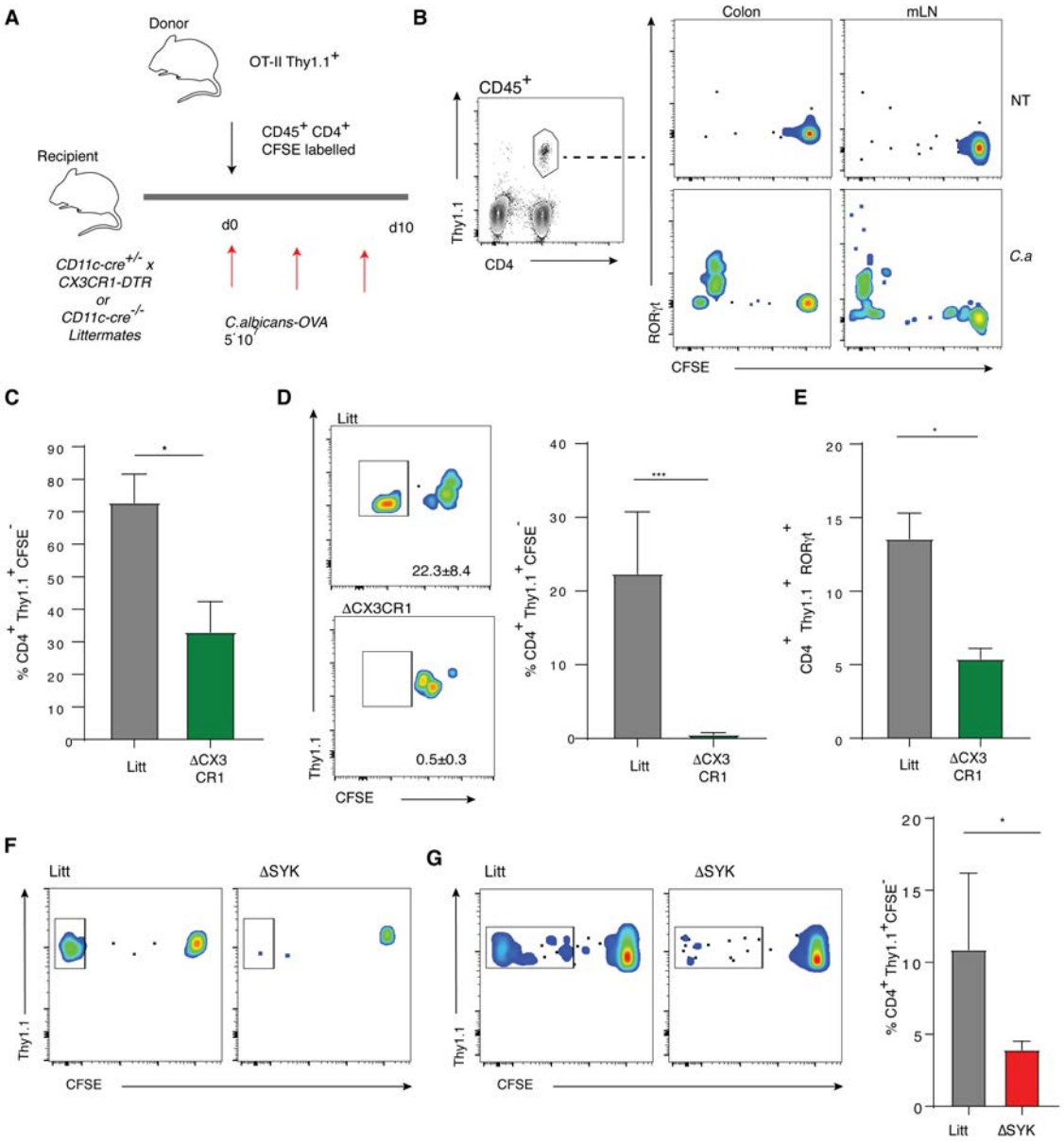

Fig. S12. Characterization of antigen specific responses towards *C. albicans* -OVA.
(A) Mice were transferred with purified CD4$^+$ Thy1.1$^+$ OT-II cells, fed *C. albicans*-OVA and sacrificed at day 10. **(B)** CD4$^+$ Thy1.1$^+$ OT-II cells proliferation and RORγ expression in the colon (left) and mLNs (right) of control mice (not treated, NT) or mice fed with *C. albicans*-OVA. (**C**) Quantification of CD4$^+$ Thy1.1$^+$ OT-II cells proliferation in the colon of ΔCX3CR1 mice or wild type littermates. (**D**) CD4$^+$ Thy1.1$^+$ OT-II cells proliferation in the mLN of ΔCX3CR1 mice or wild type littermates. (**E**) Antigen specific Th17 responses in the colon of ΔCX3CR1 mice or WT mice. **(F, G)** ΔSYK mice and controls were transferred with CD4$^+$ Thy1.1$^+$ OT-II cells, fed *C. albicans*-OVA and sacrificed at day 10. CD4$^+$ Thy1.1$^+$ OT-II cells proliferation in colon **(F)** and mLNs **(G)**. Data expressed as mean ± SEM of individual mice, representative of three independent experiments.

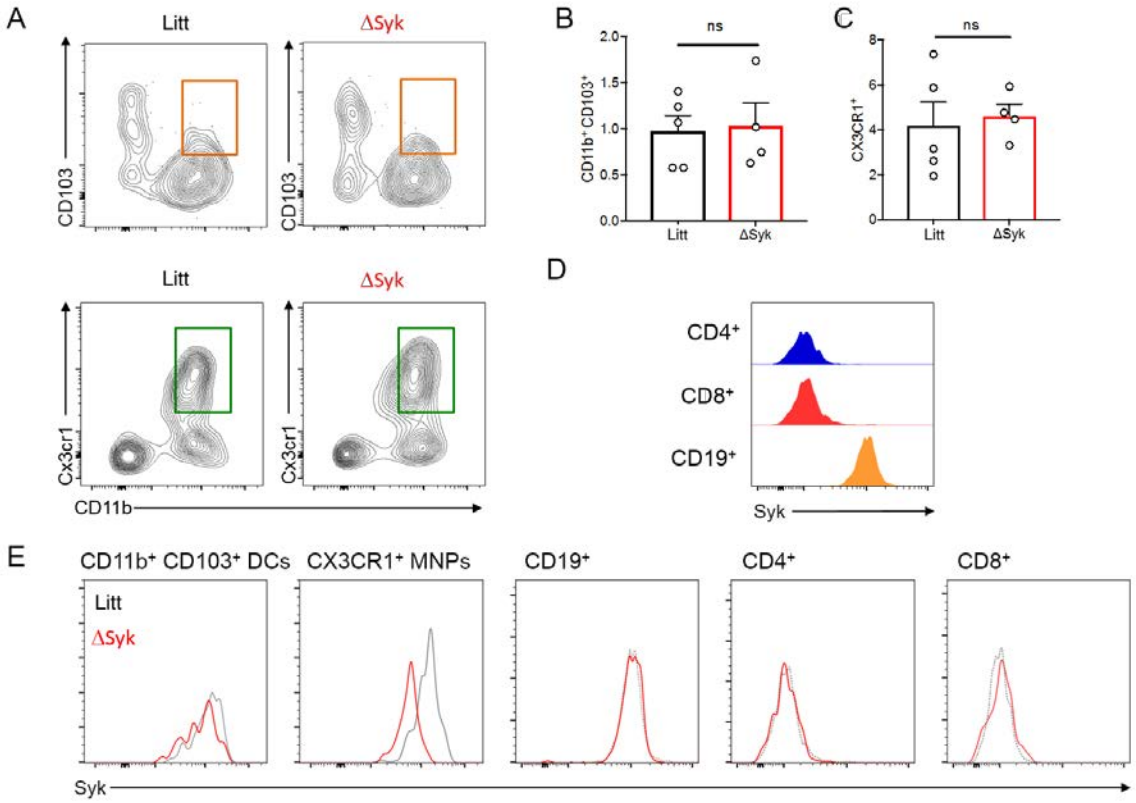

Fig. S13. Specific depletion of Syk in CX3CR1^{+} cells.

(A) Representative flow cytometry plot of the colonic, Lin^{-}CD45^{+}MHC-II^{+}CD11c^{+} lymphocytes in ΔSyk mice (*Cx3cr1-cre/ERT2*$^{+/-}$ *Syk*$^{fl/fl}$ mice) mice or wild type (*Cx3cr1-cre/ERT2*$^{-/-}$ *Syk*$^{fl/fl}$ mice) littermates. Quantification of Lin^{-} CD45^{+}MHC-II^{+} CD11c^{+} **(B)** CD11b^{+} CD103^{+} DCs and **(C)** CD11b^{+} CX3CR1^{+} MNPs among CD45^{+} cells. **(D)** Representative flow cytometry plot of Syk expression by colonic CD4^{+} T cell, CD8^{+} T cells and CD19^{+} B cells in C57BL6 WT mice; Syk is highly expressed among B cells but not among CD4^{+} T cell, CD8^{+} T cells **(E)** Representative flow cytometry plot of Syk expression among CD11b^{+} CD103^{+} DCs, CD11b^{+} CX3CR1^{+} MNPs, CD4^{+} T cell, CD8^{+} T cells and CD19^{+} B in ΔSyk mice (*Cx3cr1-cre/ERT2*$^{+/-}$ *Syk*$^{fl/fl}$ mice) mice or wild type (*Cd11c-cre*$^{-/-}$ *Irf4*$^{fl/fl}$ mice) littermates. Syk expression was reduced upon Tamoxifen treatment in CD11b^{+} CX3CR1^{+} MNPs but not in CD19^{+} B cells, the other high Syk expressing cell type, confirming the selectivity of Syk depletion. Data expressed as mean ± SEM of individual mice, representative of three independent experiments.

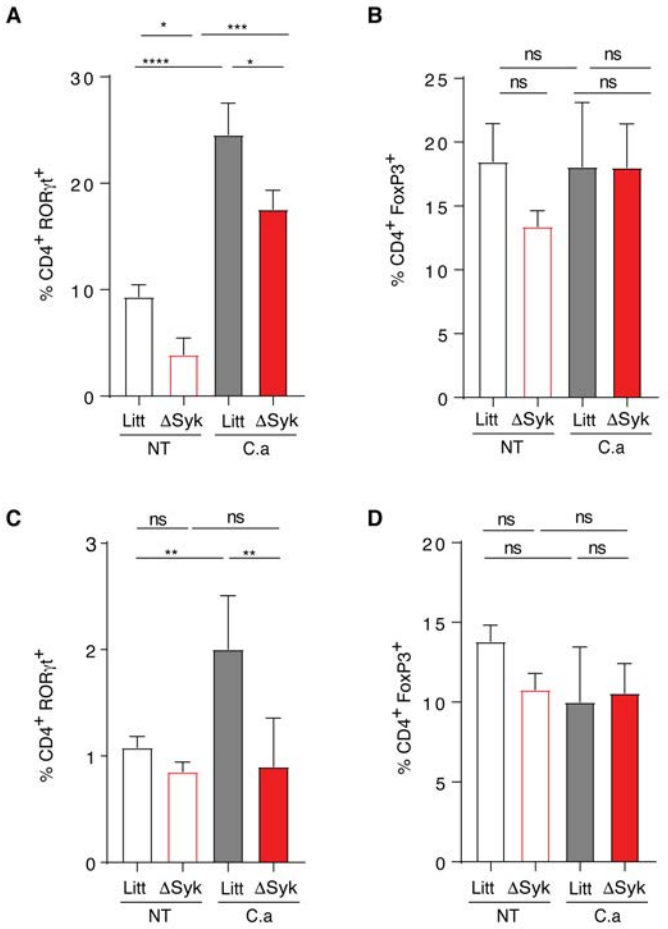

Fig. S14. Quantification of Th17 and Treg cells in the intestine of ΔSyk mice following *C. albicans* colonization.

Quantification of RORγt$^+$ Th17 **(A)** and FoxP3$^+$ Treg cells **(B)** among the CD4$^+$ T cells in the colon of ΔSyk mice or wild type littermates. Quantification of RORγt$^+$ Th17 **(C)** and FoxP3$^+$ Treg cells **(D)** among the CD4$^+$ T cells in the mLN of ΔSyk mice or wild type littermates. Data expressed as mean ± SEM of individual mice, representative of two independent experiments.

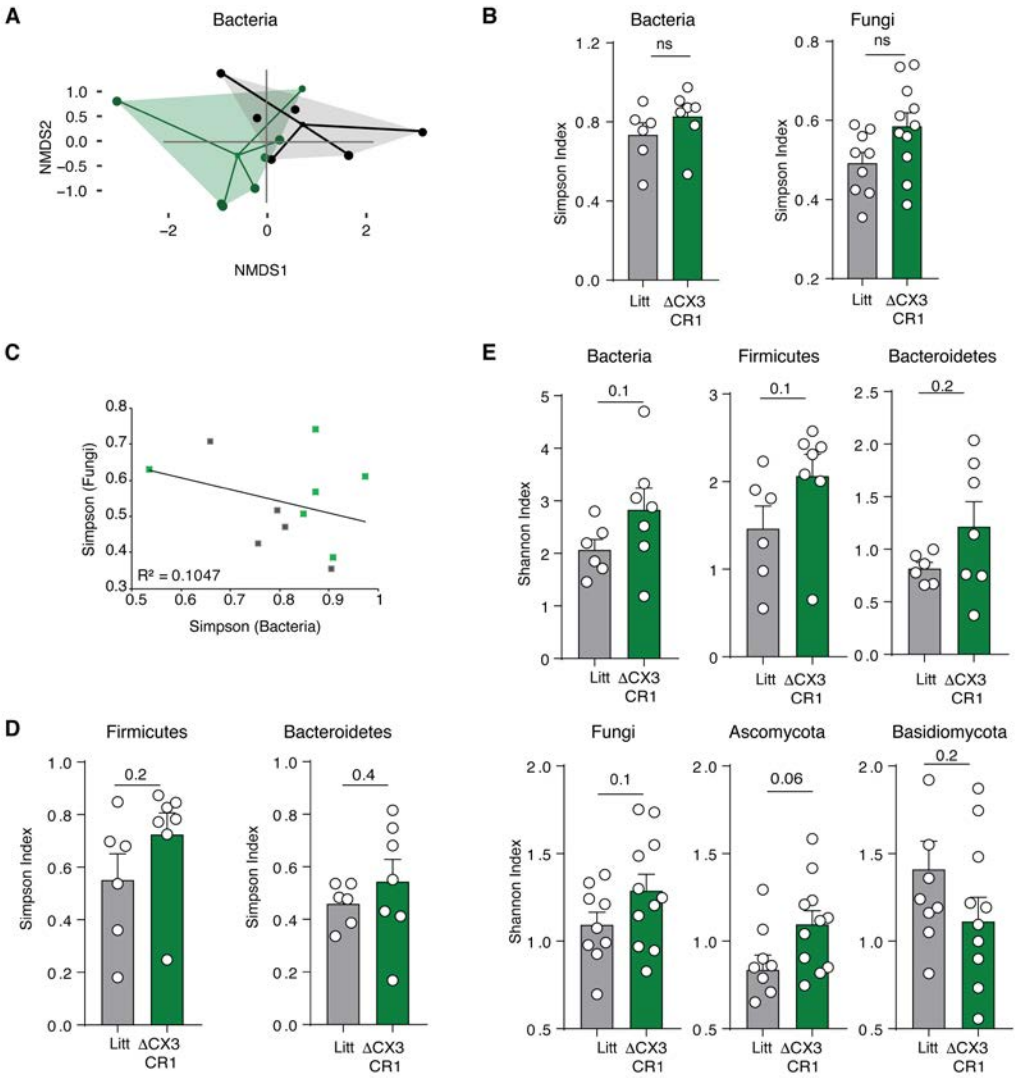

Fig. S15. Characterization of the fungal communities in the feces following CX3CR1^{+} MNPs depletion. Feces from ΔCX3CR1 or WT littermates mice were collected 7 days after administration of the first DT dose. (**A**) NMDS plot of distance ordination based on Bray-Curtis dissimilarities in the colon for bacterial OTUs. (**B**) Alpha diversity as computed using the Simpson diversity index among bacteria (left panel) and fungi (right panel). (**C**) Correlation between Simpson Diversity in the fungal community (left) or in the Ascomycota phylum (right) with the alpha diversity in the bacterial community. Of note, the increased diversity of the fungal community was independent from bacterial diversity. (**D**) Simpson diversity in the two major bacterial phyla (Firmicutes and Bacteroidetes). (**E**) Shannon diversity index among total bacteria, Firmicutes, Bacteroidetes, total fungi, Ascomycota and Basidiomycota. Statistic: data are pooled from two independent experiments, graphs show mean +/- SEM, each circle denotes one mouse. Data expressed as mean ± SEM (Mann-Whitney Test).

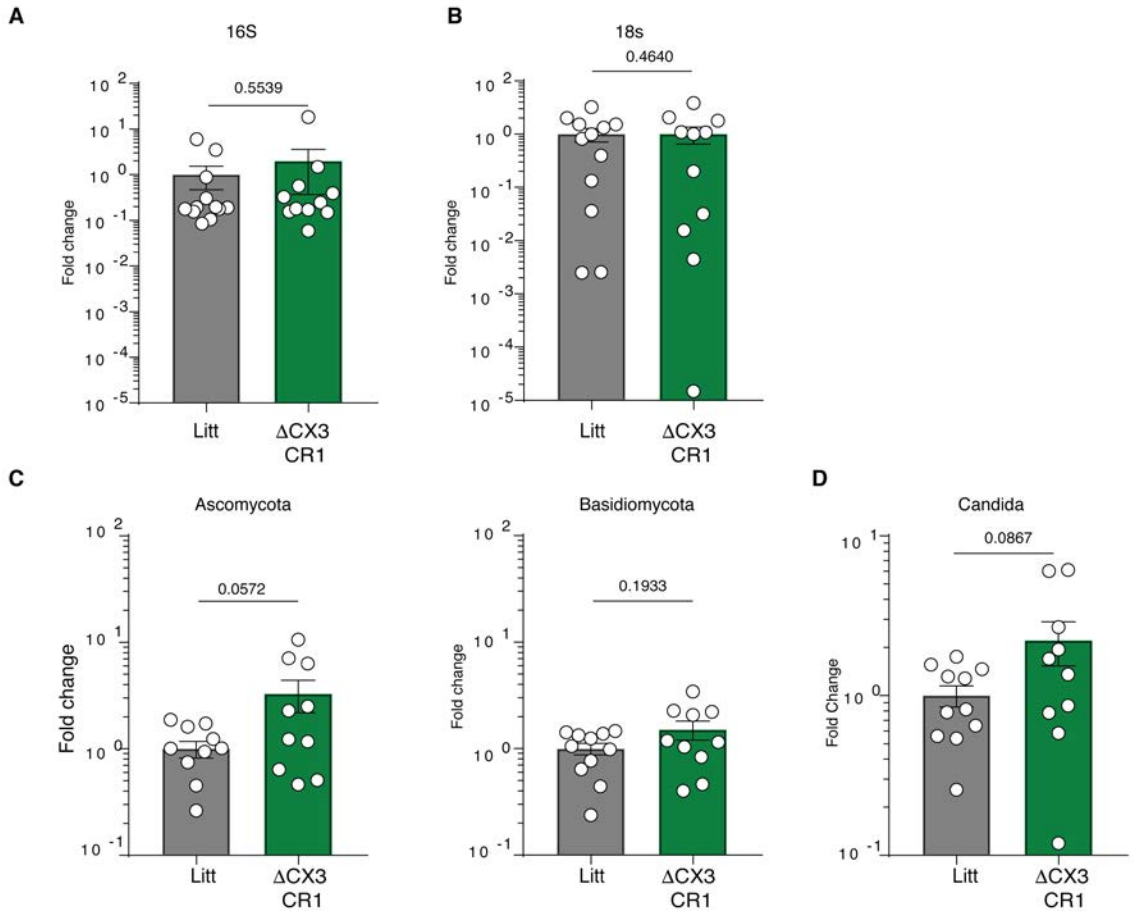

Fig S16. RT-qPCR assessment of the microbiota following CX3CR1+ MNPs depletion. (**A**) Abundance of bacteria as determined by 16S qPCR in mice following depletion of $CX3CR1^{+}$ MNPs; $2^{-\Delta CT}$ relative to 16S abundance in WT ($CD11c\text{-}cre^{-}$ littermates). **(B)** Relative abundance of fungi (18S) in ΔCX3CR1 mice relative to WT littermates after normalization to total bacteria (16S). Relative abundance of Ascomycota (**C**, left panel), Basidiomycota (**C**, right panel) and Candida (**D**) in ΔCX3CR1 mice relative to WT littermates after normalization to total fungi (18S). Data expressed as mean ± SEM, dot represent individual mice. Data are representative of two independent experiments with 4-5 mice per experiment. **P*<0.05 (Mann Whitney Test).

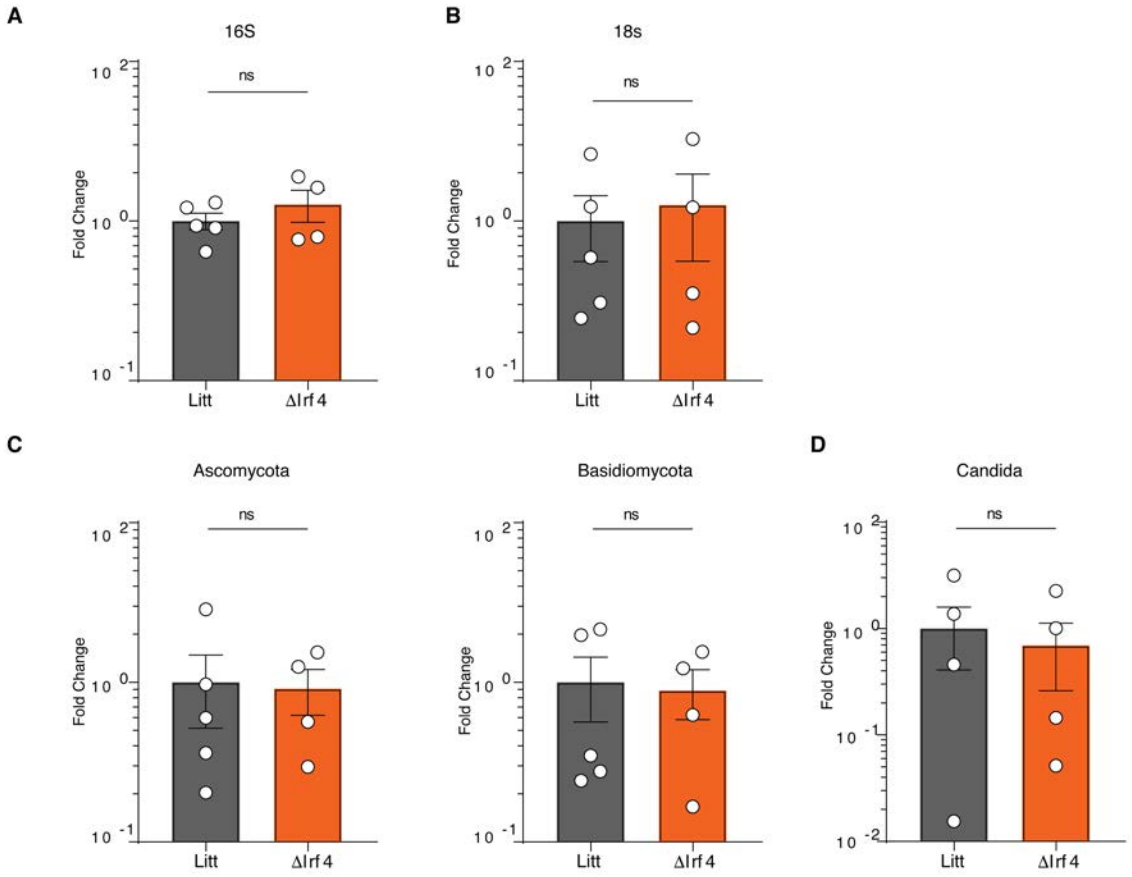

Fig. S17. RT-qPCR assessment of the microbiota in ΔIrf4 mice.

(A) Abundance of bacteria as determined by 16S qPCR in ΔIrf4 (n=4) mice relative to 16S abundance in control littermates (Litt, n=5). **(B)** Relative abundance of fungi (18S) in ΔIrf4 mice relative to WT littermates after normalization to total bacteria (16S)**.** Relative abundance of Ascomycota (**C**), Basidiomycota (**D**) and Candida (**E**) in ΔIrf4 mice relative to WT littermates after normalization to total fungi (18S). Data expressed as mean ± SEM, dot represent individual mice. Data are representative of two independent experiments with 4-5 mice per experiment. **P*<0.05, ns: not significant (Mann Whitney Test).

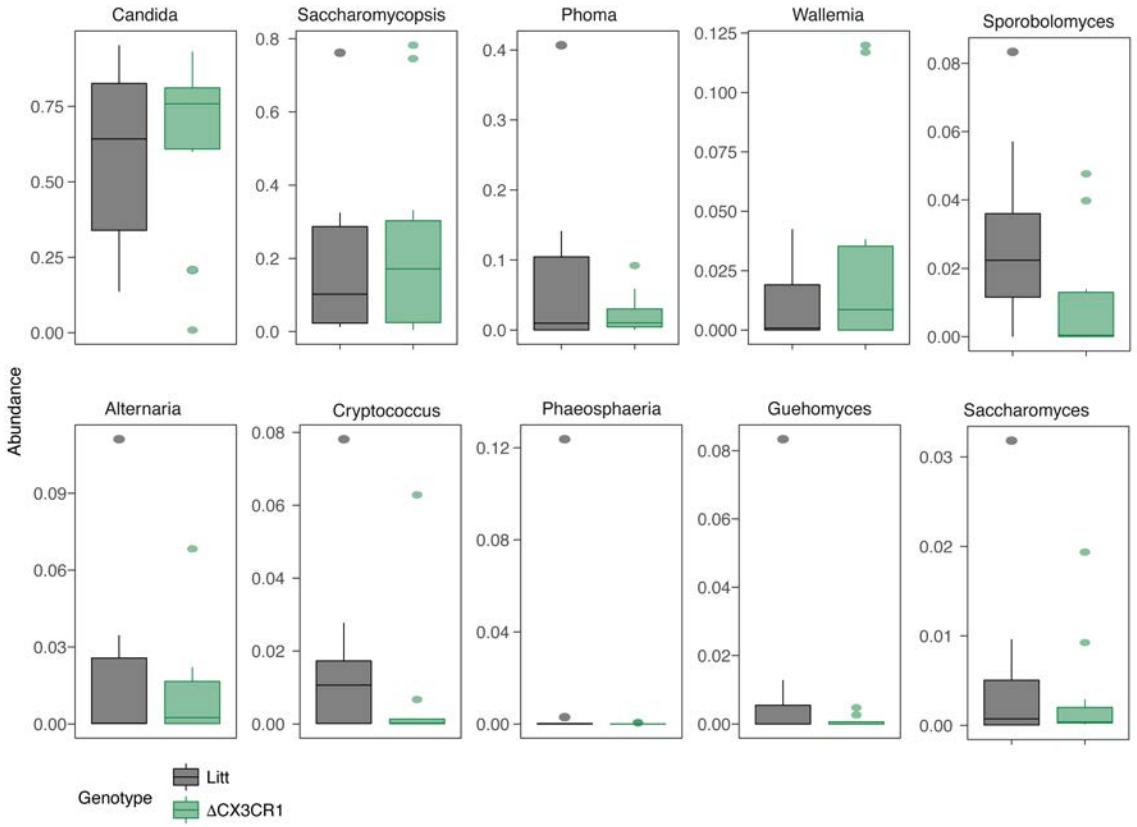

Fig. S18. Relative abundance of the 10 most abundant fungal genera in the feces of ΔCX3CR1 mice or control littermates (Litt).

Boxplot represent median and lower and upper hinges correspond to the 25th and 75th percentiles. Data are pooled from 2 independent experiments.

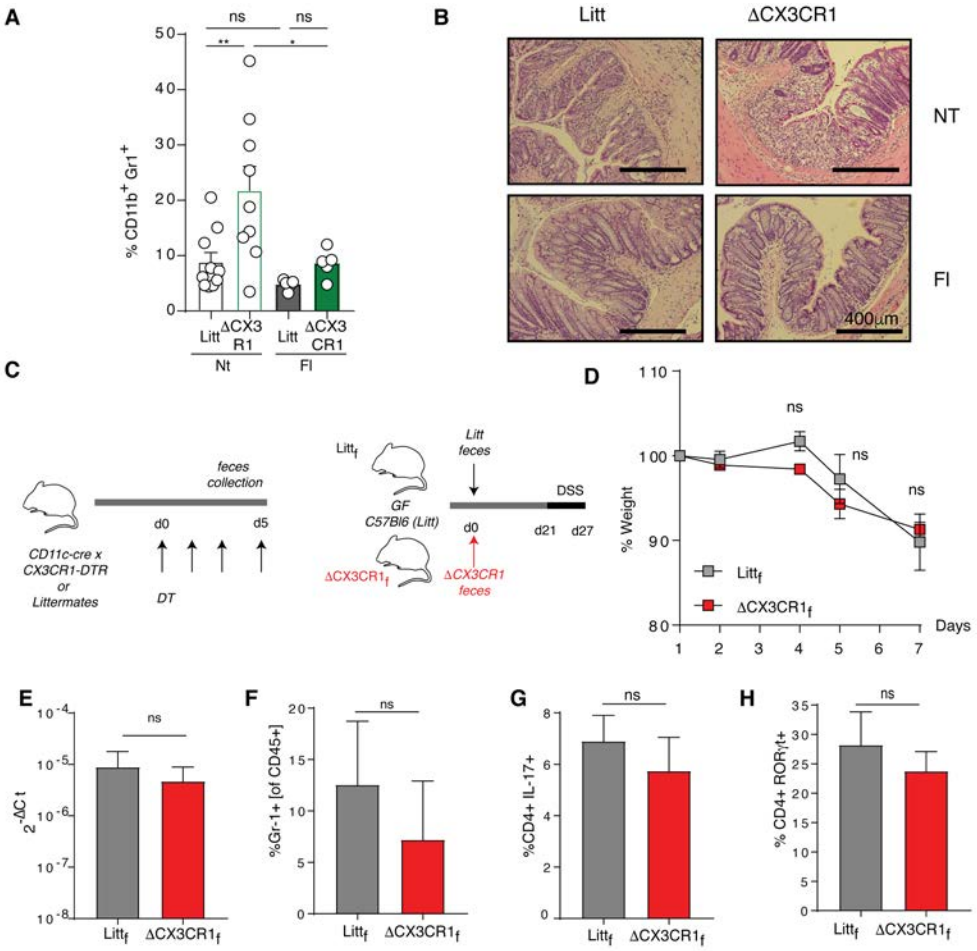

Fig. S19. Impaired antifungal-immunity increases susceptibility of ΔCX3CR1 mice to DSS colitis.

(A) DSS colitis was induced in ΔCX3CR1 or control (Litt) littermates following treatment with fluconazole (Fl) or no treatment (Nt), and neutrophil infiltration ($CD45^+$ $CD11b^+$ *Gr-1*^{high}) in the colon was assessed (pooled from two independent experiments). **(B)** Representative H&E staining of colon tissue. **(C)** Outcome of DSS colitis in germ free (GF) C57BL6 WT mice transferred with feces from ΔCX3CR1 mice or control littermates both treated with diphtheria toxin for 5 days. **(D)** Weight loss from baseline at day 21, X-axis shows day after start of DSS treatment. **(E)** Total 16s DNA in the feces of the recipient mice at day 27 (day of sacrifice). (**F**) Frequency of colonic Neutrophils (Live, $CD45^+$ $CD11b^+$ $Gr1^+$) among $CD45^+$ Lymphocytes. (**G**) Frequency of IL-17 producing $CD45^+$ $CD4^+$ cells. (**H**) Frequency of $ROR\gamma t^+$ $CD45^+$ $CD4^+$ T cells. Data expressed as mean ± SEM of individual mice (n=4-5), representative of two independent experiments. Statistical analysis: **P*<0.05, ***P*<0.01, ****P*<0.001 (Mann-Whitney Tes**t).**

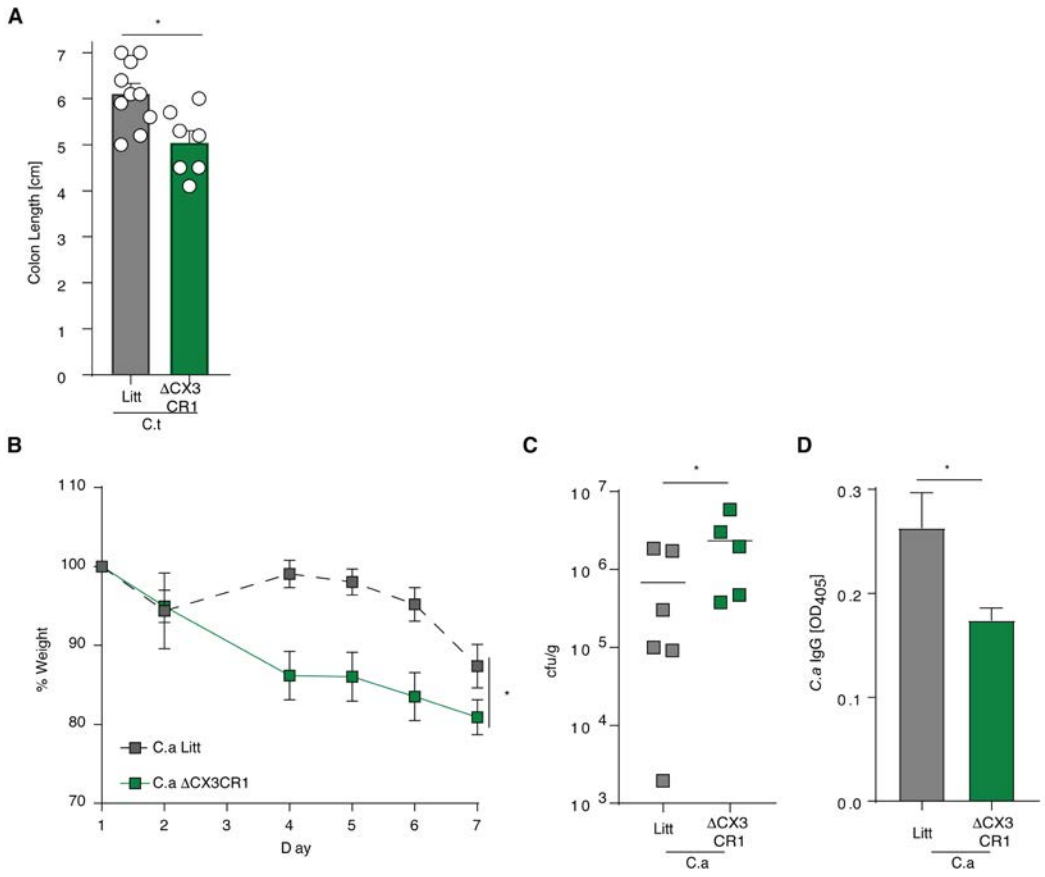

Fig. S20. Depletion of CX3CR1^{+} MNPs results in exacerbated DSS-induced intestinal disease following colonization with *Candida.*

(A) Shortening of the colon in ΔCX3CR1 or control littermates fed with *C. tropicalis* (C.t) after induction of DSS colitis. **(B)** Percent weight loss in ΔCX3CR1 or control (Litt) littermates fed with *C. albicans* (C.a) following DSS administration. **(C)** *C. albicans* was quantified by SDB plating of faecal material and expressed as cfu/g. **(D)** Systemic IgG responses against *C. albicans* were assessed by ELISA.Dots represent individual mice. **P*<0.05. Mann Whitney Test (B, C), two-way ANOVA (A).

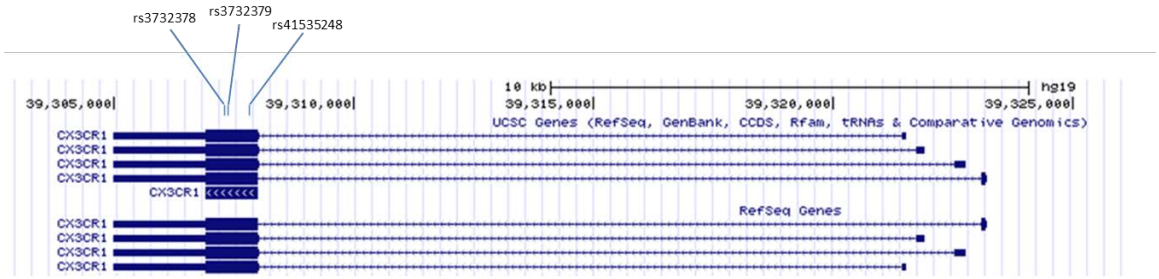

Fig. S21. Map of *CX3CR1* gene region and 3 SNPs in the coding region.

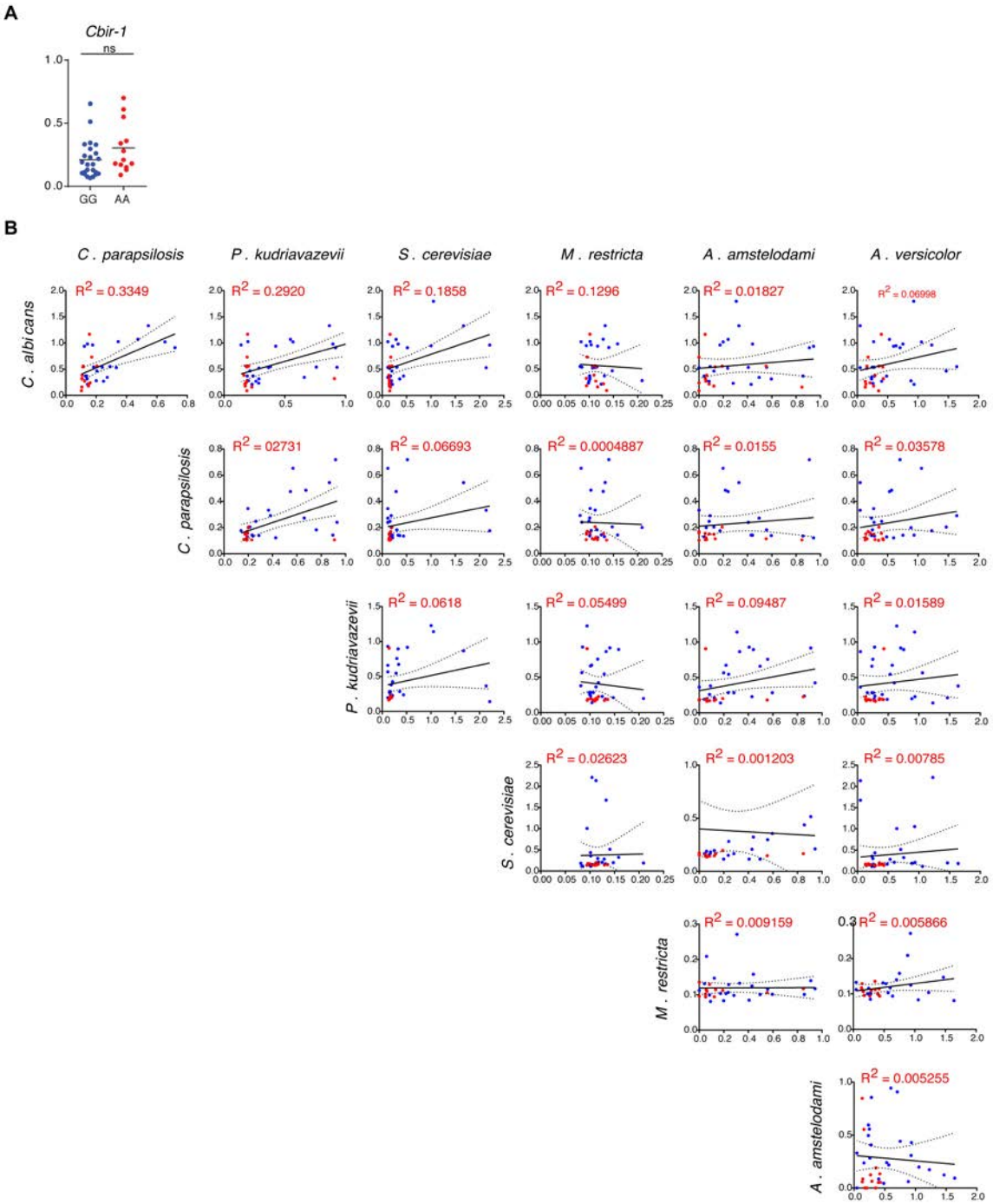

Fig. S22. Human serum IgG responses

(**A**) IgG responses against cbir. (**B**) Linear correlation between IgG responses against different fungal species**.** Values in red represent R^2 value from the Pearson correlation coefficient. Weak correlation coefficient suggests lack of serum antibody cross-reactivity between the respective fungi. Red dots: AA patients, blue dots GG patients.

Table S1.
Association of missense mutations in *CX3CR1* with Crohn's Disease (CD)

rsID	CHR	Position*	FA[#]	FU[$]	OR	L95	U95	P
rs3732378	3	39307162	0.18	0.17	1.04	0.97	1.12	0.25
rs3732379	3	39307256	0.29	0.28	1.05	0.99	1.11	0.15
rs41535248	3	39307962	0.01	0.01	1.08	0.84	1.39	0.54

*: position in HG19;
[#]: Minor Allele Frequency (MAF) in CD cases;
[$]: MAF in non-IBD controls

Table S2.

Association of missense mutations in *CX3CR1* with serological markers in CD patients

SNP	serology	FA*	FU$	OR	L95	U95	P
	anca	0.181	0.174	1.13	0.77	1.64	0.535
	cbir	0.165	0.182	0.88	0.63	1.23	0.459
rs3732378	i2	0.159	0.202	0.69	0.48	0.98	0.041
	igg.asca	0.135	0.205	0.59	0.41	0.84	3.73E-03
	anca	0.290	0.287	1.05	0.77	1.44	0.750
	cbir	0.284	0.289	0.98	0.74	1.29	0.874
rs3732379	i2	0.277	0.307	0.83	0.62	1.12	0.218
	igg.asca	0.260	0.307	0.79	0.59	1.05	0.103
	anca	0.015	0.009	1.53	0.44	5.40	0.505
	cbir	0.006	0.015	0.42	0.11	1.62	0.208
rs41535248	i2	0.012	0.006	2.23	0.51	9.79	0.287
	igg.asca	0.017	0.007	2.60	0.75	9.05	0.133

*: MAF in serology positive CD patients;

$: MAF in serology negative CD patients;

Movie S1

A confocal microscopy video showing a 3D rendering of the colonic lamina propria of *Cx3cr1*$^{GFP/+}$ mice fed with *C. albicans*-RFP, as shown in Figure 1D. CX3CR1^{+} cells are shown in green, DAPI nuclear staining in blue, *C. albicans*-RFP in red.

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