Supplementary Materials for

DNGR-1 in DCs limits tissue damage by dampening neutrophil recruitment

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This PDF file includes:

Materials and Methods
Figs. S1 to S13

Materials and Methods

Reagents and *Candida albicans*

Reagents used were as follows: Caerulein (Sigma), anti-DNGR-1 blocking antibody (7H11, BioXCell), anti-Ly6G antibody (1A8, BioXCell) and their polyclonal rat IgG isotype controls (Sigma); Fungizone (Amphotericin B, Whittaker Bioproducts); CpG (ODN 1668, Sigma), Whole Glucan Particles (WGP, Biothera), NSC-87877 (SHP inhibitor, Calbiochem), pepducin and control peptides (Genescript) and cefovecin antibiotic (Zoetis). High-binding ligand for DNGR-1 (DNGR-1L) is a combination of F-actin and myosin II kindly provided by Dr. C. Reis e Sousa (*20*).

Candida albicans (strain SC5314, kindly provided by Prof. C. Gil, Complutense University, Madrid) was grown on YPD-agar plates (Sigma) at 30ºC for 48h. Heat-killed *C. albicans* (HKC) was prepared by boiling for 30 minutes.

Mouse strains and cells

Mouse colonies (6 – 12 weeks old), were all on a C57BL/6 background and bred at CNIC under specific pathogen-free conditions. They included WT, *Clec9a*$^{gfp/gfp}$ (DNGR-1-deficient, B6(Cg)-Clec9atm1.1Crs/J) (*7*), *Batf3*$^{-/-}$ (B6.129S(C)-*Batf3*^{tm1Kmm}/J, The Jackson Laboratory), *Rag1*$^{-/-}$ *Clec9a*$^{gfp/gfp}$, which were generated by crossing *Rag1*$^{-/-}$ (B6.129S7-Rag1tm1Mom/J, The Jackson Laboratory) with *Clec9a*$^{gfp/gfp}$ mice; *Ptpn6*$^{fl/fl}$*Itgax*$^{+/+}$ and *Ptpn6*$^{fl/fl}$*Itgax*$^{Cre/+}$ kindly provided by Prof. Clifford A. Lowell, UCSF, San Francisco, CA (CD11cΔSHP-1, bred as littermates) (*28*); and *Cxcl2*$^{-/-}$*Clec9a*$^{gfp/gfp}$, which were generated by crossing *Cxcl2*$^{-/-}$ (MIP-2-deficient, C57BL/6NJ-*Cxcl2*$^{em1(IMPC)J/}$J) with *Clec9a*$^{gfp/gfp}$ mice. Mice expressing the CD45.1 allele (B6.SJL-*Ptprc*^{a}*Pepc*^{b}/BoyJ, The Jackson Laboratory) were used as recipients for mixed bone marrow chimeras. Experiments were conducted with sex- and age-matched mice. Experiments were approved by the animal ethics committee at CNIC and conformed to Spanish law under Real Decreto 1201/2005. Animal procedures were also performed in accordance to EU Directive 2010/63EU and Recommendation 2007/526/EC.

B3Z cells (kindly provided by Prof. N. Shastri, University of California, Oakland) express a β-gal reporter for nuclear factor of activated T cells (NFAT) (*7*). B3Z cells stably transduced with mouse *Clec9a* and *Syk* (B3Z-mDNGR-1-Syk cells) allow the detection of DNGR-1 ligands by testing their agonistic capacity through NFAT reporter activation and induction of β-gal activity. Cells were cultured in RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 50 µM 2-mercaptoethanol, and 10% heat-inactivated fetal bovine serum (FBS) (all from Life Technologies, Carlsbad, CA) at 37ºC and regularly checked for the absence of mycoplasma. Reporter assays were carried out in serum-free AIM-V medium (Life technologies). Non-transduced B3Z or B3Z-mDNGR-1-Syk cells were incubated with plated DNGR-1L at the indicated concentrations. If needed, cells were pre-incubated for 30 min with 20 µg/ml of 7H11 (or isotype-matched control). After overnight culture, LacZ activity was measured lysing cells in CPRG (Roche, Basel, Switzerland)-containing buffer. 1-4 hours later, O.D. 595 was measured relative to O.D. 655 nm used as a reference.

The MutuDC1940 line (MutuDCs), kindly provided by Prof. H. Acha-Orbea, (University of Lausanne, Lausanne, Switzerland) was kept in culture and harvested at ≈ 90% of confluence as described (*20*). Cells were plated on RIA plates (Corning) in the presence or not of DNGR-1L (0.1 µM) for 5 minutes and subsequently stimulated with HKC (10:1 ratio), WGP (150 µg/ml) or CpG (0.5 µg/ml) for given times. In cases without further stimulation, MutuDCs were plated with DNGR-1L for indicated times. When required, MutuDCs were pre-incubated before

stimulation for 30 min with either 20 μM of the SHP inhibitor NSC-87877, 7H11 antibody (250 μg/ml), or isotype-matched control.

In vivo models

For *Candida albicans* systemic infections, mice were intravenously (i.v.) infected with 10^5 *C. albicans* in sterile phosphate buffered saline (PBS, Life Techonologies) and monitored daily for weight, general health, and survival, following the institutional guidance. Control mice were given PBS. Fungizone was intraperitoneally (i.p.) administered (2 mg/kg body weight in PBS) daily from day 3 post-infection (p.i.) onwards. Pepducin and corresponding control peptides were subcutaneously (s.c.) injected daily, starting at day 3 p.i. Unless otherwise specified, animals were culled at day 6 p.i.

Kidney fungal burden was determined by plating organ homogenates obtained mechanically over 70-μm cell strainers (BD Biosciences) after slicing the tissue, in serial dilutions on YPD agar plates. Colony-forming units (CFU) were counted after growth at 30ºC for 48 h.

To obtain cell suspensions for flow cytometry, kidneys were digested with Liberase TL (Roche) for 10 minutes at 37ºC and filtered through 70-μm cell strainers (BD Biosciences). For the isolation of bone marrow cells (BM), femurs were collected and flushed. In all cases, red blood cells were lysed using RBC Lysis Buffer (Sigma) for 3 minutes at room temperature (RT). Washes and phenotypic analysis of leukocytes were performed on ice-cold FACS Buffer (PBS supplemented with 5 mM EDTA and 3% FBS).

For quantitative PCR analysis of Kim-1 expression, RNA was purified from total kidneys.

Neutrophil counts in blood and creatinine levels from serum samples were determined by the Biochemical Analysis unit at CNIC.

In experiments addressing cytokine expression in vivo, both kidneys from at least four mice per group were harvested at day 2.5 (60 hours) p.i. and processed as described. Leukocytes were enriched by Percoll centrifugation (Sigma) (40% in HBSS, 300 x *g*, 30 minutes, RT) and populations were isolated by cell sorting.

Caerulein-induced acute pancreatitis was induced by seven intraperitoneal injections of caerulein (50 μg/kg body weight in PBS at intervals of 1 h). Control mice were given PBS. All animals were sacrificed 12 h after the last injection of caerulein. Pancreas and BM were processed as described above. Flow cytometric analysis of pancreas immune infiltrates was also performed on cell suspensions as indicated before. Neutrophil count in blood and serum lipase levels were measured by the Biochemical Analysis unit at CNIC.

When necessary, mice were treated i.p. with 100 μg of 7H11 anti-DNGR-1 blocking (BioXCell) antibody or isotype-matched control. For acute pancreatitis, mice were injected on days -1 and 0. In the case of *C. albicans* infection, injections were administered daily from day -1 to sacrifice.

In neutrophil-depleting experiments, mice were treated i.p. with anti-Ly6G antibody (clone 1A8 (BioXCell), 25 μg per injection and mouse in PBS) or isotype-matched control. In acute pancreatitis, mice were injected on days -1 and 0. For the *C. albicans* infection model, antibodies were administered daily, starting at day 3 post-infection.

Generation of mixed bone marrow chimeras

CD45.1 lethally irradiated (5.5 Gy, twice) mice were i.v. reconstituted with up to 5 x 10^6 of total bone marrow cells in PBS consisting of 50:50 mixtures of *Batf3*$^{-/-}$:WT, *Batf3*$^{-/-}$

:*Clec9a*$^{gfp/gfp}$, *Batf3*$^{-/-}$:*Cxcl2*$^{-/-}$ or *Batf3*$^{-/-}$:*Cxcl2*$^{-/-}$ *Clec9a*$^{gfp/gfp}$. Mice were subcutaneously injected with cefovecin (30 mg/kg body weight in PBS). Blood was collected 3 weeks later, lysed and stained on ice-cold FACS Buffer to check reconstitution. One week later, mice were i.v. infected with 7 x 10^4 *C. albicans*, due to increased susceptibility to the fungus of *Batf3*$^{-/-}$:WT control chimeric mice compared with regular (non-irradiated) WT mice. Mice were monitored and culled as described above.

Antibodies and flow cytometry

Samples were stained with the appropriate antibody cocktails in ice-cold FACS Buffer. Antibodies included biotinylated anti-DNGR-1 (7H11), Alexa647-anti-Dectin-1 (2A11, AbD Serotec, Kidlington, UK), FITC-anti-Ly6C (AL-21), FITC or PECy7-anti-CD11b (M1/70), PeCy7, PercPCy5.5 or APC-anti-CD11c (HL3), PE-anti-Ly6G (1A8) and Brilliant Violet 421-anti-B220 (RA3-6B2) from BD Pharmingen (San Diego, CA); PercPCy5.5-anti-CD45 (30-F11), PercPCy5.5-anti-CD45.2 (104), APC-anti-CD45.1 (A20) and APC or PE-streptavidin from eBioscience (Hatfield, UK); Violet Fluor 450 anti-CD3 (17A2) from TONBO Bioscience (San Diego, CA) and PE-anti-XCR1 (ZET) from Biolegend (San Diego, CA). Dead cells were excluded by Hoechst 33258 (Invitrogen, Carlsbad, CA) incorporation. Purified anti-FcγRIII/II (2.4G2, TONBO Bioscience) was used to block Fc-receptors at 4ºC for 10 minutes in all the stainings. Regarding immune infiltration analysis, monocytes were defined as Hoechst$^-$ CD45$^+$CD11b$^+$Ly6C$^{+/bright}$Ly6G$^-$ and neutrophils as Hoechst$^-$CD45$^+$CD11b$^+$Ly6C$^+$Ly6G$^+$. Reconstitution of mixed bone marrow chimeras was assessed by checking CD45 allotype of neutrophils. Events were acquired using LSRFortessa flow cytometer (BD).

For cell sorting, Percoll-enriched leukocytes were labelled with FITC-anti-CD19 (1D3), Alexa 647-anti-CD3 (17A2), PeCy7-anti-CD11c (HL3), APCCy7-anti-CD11b (M1/70), FITC-anti-Ly6C, PercPCy5.5-anti-SiglecF (E50-2440), PE-anti-Ly6G and Alexa 647-anti-CD64 (X54-5/7.1) from BD Pharmingen; Brilliant Violet 570-anti-CD45 (30-F11) and PE-anti-XCR1 from Biolegend; PercPCy5.5-anti-CD127 (A7R34) and PE-anti-NK1.1 (PK136) from TONBO Bioscience; biotinylated anti-CD172α (Sirpα) (P84), PercPCy5.5-streptavidin from eBioScience and Hoechst 33258. It was followed by flow cytometric sorting of CD45$^+$CD11b$^+$Ly6G$^+$ (neutrophils), CD45$^+$CD11b$^+$Ly6G$^-$CD64$^+$ (macrophages), CD45$^+$CD11b$^-$CD11c$^+$XCR1$^+$ (cDC1s), CD45$^+$CD11b$^+$Ly6G$^-$CD64$^-$CD11c$^+$Sirpα$^+$ (cDC2s), CD45$^+$CD11b$^+$Ly6G$^-$CD64$^-$ Sirpα$^-$Ly6C$^{+/bright}$ (monocytes), CD45$^+$CD3$^+$ (T cells), CD45$^+$CD19$^+$ (B cells), CD45$^+$CD3$^-$ CD19$^-$NK1.1$^+$ (NK cells), CD45$^+$ CD3$^-$CD19$^-$NK1.1$^-$CD127$^+$ (ILCs) and CD45$^+$Ly6G$^-$SiglecF$^+$ (eosinophils), Hoechst$^-$ populations (see fig. S10) on FACSAria SORP (BD) or Sy3200 (Sony Biotechnology) flow sorters. Data were analyzed with FlowJo software (Tree Star).

Histopathology and TUNEL staining

Kidneys or pancreas were fixed in 4% PFA (Thermo Fisher Scientific, Walthman, MA) until processed by the Histopathology unit at CNIC. Samples were dehydrated with ethanol, embedded in paraffin and sections (3-5-µm thickness) were stained with hematoxylin and eosin (H&E, Sigma) following standard protocols. Tissue sections were visualized under a Leica DM2500 microscope (Leica Microsystems, Solms, Germany) and quantification was performed using ImageJ software (Bitplane, Belfast, UK).

For cell death assay by TUNEL staining, renal samples were fixed with 4% PFA and embedded in O.C.T. (Tissue-Tek, Torrance, CA) for cryosectioning. *In situ* detection of cells with DNA-strand breaks was performed on sections (5 µm) by the TUNEL (terminal

deoxynucleotydil transferase dUTP nick end labelling) staining method using the In Situ Cell Death Detection Kit (Roche) according to manufacturer's instructions. Sections were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI, Eugene, OR). Confocal images were obtained with a Leica TCSSP5 confocal scanning laser unit attached to an inverted epifluorescence DMI6000B microscope fitted with an HCX PL APO lambda blue 63X/1.4 NA oil immersion objective, using Las-AF acquisition software (Leica). For image processing and analysis, two channels were used for the analysis: C1_DAPI (blue) and C2_TUNEL (red). A total of 150 images with two channels each were processed by using custom-designed algorithms within Definiens Developer XD software (Definiens AG, München, Germany). TUNEL positive nuclei were determined by applying a red threshold of 80. Every nucleus showing a mean value of the C2_TUNEL superior to 80, in a ring region with a dilation of two pixels, was classified as positive. Percentage of TUNEL positive cells was calculated by plotting TUNEL positive cell number against total cell number (DAPI positive). This processing was performed by the Cellomics Unit at CNIC.

Quantitative-PCR

RNeasy Plus Mini Kit or RNeasy Micro Kit (in case of cell sorting), both from Qiagen (Hilgen, Germany), were used for RNA extraction. cDNA was prepared using the High Capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Quantitative PCR was performed in a 7900-FAST-384 instrument (Applied Biosystems) by using the GoTaq qPCR master mix from Promega (Madison, WI). Primers used in this work (synthetized by Sigma) were as follows: β-actin Fw: 5′-GGCTGTATTCCCCTCCATCG-3′; β-actin Rv: 5′-CCAGTTGGTAACAATGCCATGT-3′; KIM-1 Fw: 5′-CATATCGTGGAATCACAACGAC-3′; KIM-1 Rv: 5′-ACAAGCAGAAGATGGGCATTG-3′; TNFα Fw: 5′-CCCTCACACTCAGATCATCTTCT-3′; TNFα Rv: 5′-GCTACGACGTGGGCTACAG-3′; MIP-2 Fw: 5′-ACGCCCCCAGGACCC-3′; MIP-2 Rv: 5′-CTTTTTGACCGCCCTTGAGA-3′; EGR-2 Fw: 5′-GTGCCAGCTGCTATCCAGAAG-3′; EGR-2 Rv: 5′-GGCTGTGGTTGAAGCTGGAG-3′. mRNA levels were normalized to β-actin expression. Data are shown as fold induction or relative expression to β-actin (ΔΔCt) as indicated in figure legends.

Immunoblot

Cell lysates were prepared in RIPA buffer containing protease and phosphatase inhibitors (Roche). Samples were run on Mini-PROTEAN TGX PRECAST Gels and transferred onto a nitrocellulose membrane (both from Bio-Rad Laboratories) for blotting with the following antibodies: β-actin (C4) and SHIP-1 (P1C1) from Santa Cruz; PLCγ2 (MAB3716-SP) from R&D Systems; P- PLCγ2 (Tyr759, #3874S), IκB (#9242S), P-SHP-1 (Tyr564, #8849), SHP-1 (#3759), P-SHIP-1 (#3941S) and LYN (#2732), all from Cell Signaling. Alexa Fluor-680- (Life Technologies) or Qdot-800-conjugated (Rockland) secondary antibodies were used and gels were visualized in an Odyssey instrument (LI-COR).

Statistical Analysis

All statistical analyses were performed using Prism software (GraphPad Software). Statistical significance for comparison between two sample groups with a normal distribution (Shapiro–Wilk test for normality) was determined by unpaired two-tailed Student's *t*-test, unless indicated otherwise. In case not following a Gaussian distribution, statistical significance was

established by nonparametric Mann–Whitney test. Comparison of survival curves was carried out by Log-rank (Mantel–Cox) test. For comparison of weight loss evolution, two-way ANOVA tests were performed. Outliers were identified by means of Tukey's range test. Differences were considered significant at $p < 0.05$ (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). No asterisk indicates non-significant, with some non-significant p-values indicated.

In figure legends, "N" represents the number of independent experiments performed and "n" the number of individual mice included per experiment as indicated.

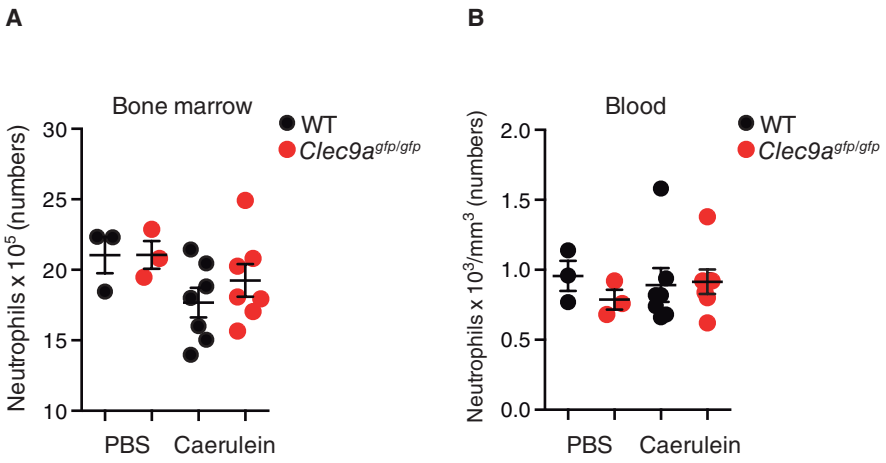

Fig. S1. DNGR-1 does not impact neutrophil count in bone marrow and blood during acute pancreatitis.

Acute pancreatitis was induced by seven intraperitonal injections of caerulein (50 micrograms/Kg) hourly for 6 hours. PBS injection was used as control. Animals were sacrificed 12 hours after the last injection. Neutrophil count in bone marrow (**A**) (N=3) and blood (**B**) (N=2) were determined in WT and *Clec9a*$^{gfp/gfp}$ mice. Each dot represents a single mouse (n=3-7). Mean±SEM of a representative experiment.

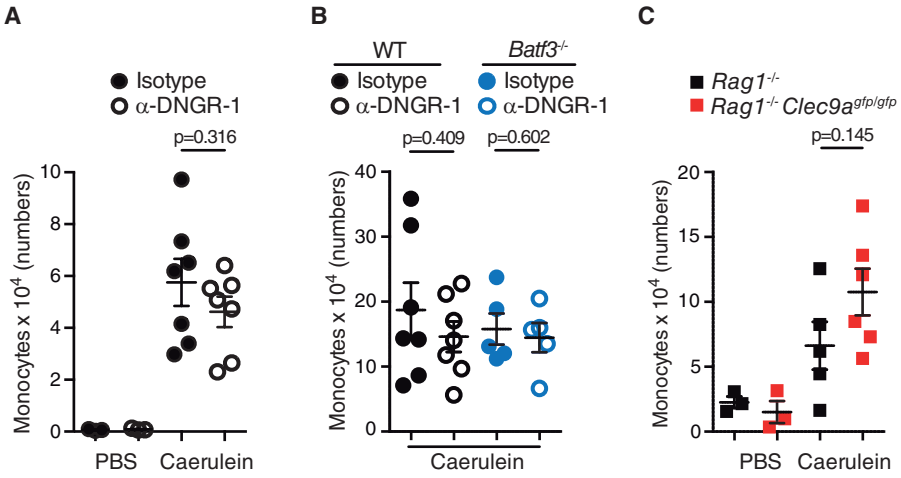

Fig. S2. DNGR-1 does not affect infiltrated monocytes during acute pancreatitis.

Acute pancreatitis was induced by seven intraperitonal injections of caerulein (50 micrograms/Kg) hourly for 6 hours. PBS injection was used as control when indicated. Animals were sacrificed 12 hours after the last injection. Anti-DNGR-1 or isotype control antibodies were intraperitoneally injected on days -1 and 0 in (**A**) WT or (**B**) WT and *Batf3*$^{-/-}$ mice. Infiltrated monocytes were assessed in pancreas by flow cytometry. (N=2) (**C**) *Rag1*$^{-/-}$ and *Rag1*$^{-/-}$*Clec9a*$^{gfp/gfp}$ mice were treated with PBS or caerulein as indicated and pancreatic infiltrating monocytes were quantified by flow cytometry (N=3). Each dot represents a single mouse (n=3-7). Mean±SEM of a representative experiment. Significance was assessed by unpaired Student's *t*-test between genotypes (C) or treatments (A, B).

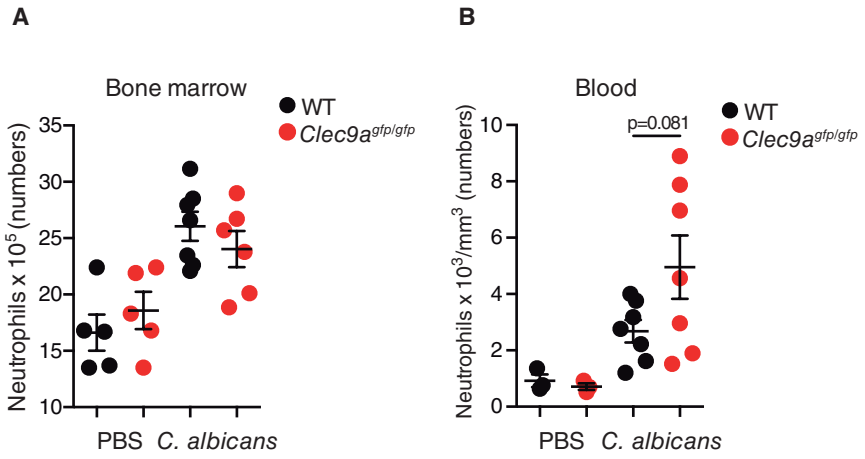

Fig. S3. DNGR-1 does not significantly impact neutrophil count in bone marrow and blood during systemic *Candida albicans* infection.

Mice were intravenously infected with 10^5 *Candida albicans*. After 6 days, neutrophil count in bone marrow (**A**) and blood (**B**) were determined in WT and *Clec9a*$^{gfp/gfp}$ mice. Each dot represents a single mouse (n=3-7). Mean±SEM of a representative experiment (N=2). Significance was assessed by unpaired Student's *t*-test between genotypes.

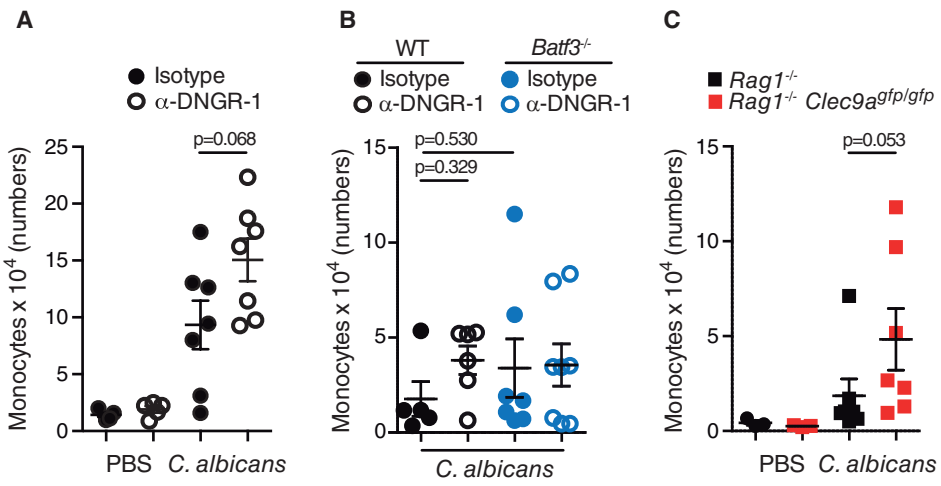

Fig. S4. Monocyte recruitment into *Candida albicans*-infected kidneys is not significantly affected under DNGR-1-deficient conditions.

Mice were intravenously infected with 10^5 *Candida albicans* and infiltrating renal monocytes were analyzed by flow cytometry after 6 days. (**A, B**) anti-DNGR-1 or isotype control antibodies were intraperitoneally injected in WT (A) (N=3) or WT and *Batf3*$^{-/-}$ (B) (N=2) mice on day -1 and daily after infection. (**C**) *Rag1*$^{-/-}$ and *Rag1*$^{-/-}$*Clec9a*$^{gfp/gfp}$ mice were infected as indicated (N=3). Each dot represents a single mouse (n=3-7). Mean±SEM of a representative experiment. Significance was assessed by unpaired Student's *t*-test between genotypes (C) or treatments (A, B).

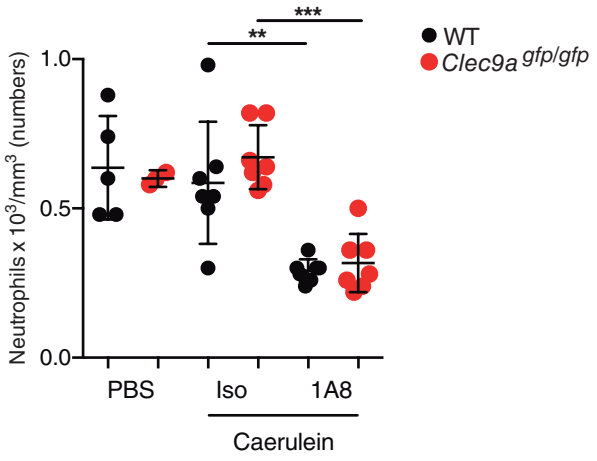

Fig. S5. Efficient 1A8-mediated neutrophil depletion during acute pancreatitis.

Acute pancreatitis was induced by seven intraperitonal injections of caerulein (50 µg/Kg) hourly for 6 hours. PBS injection was used as control. On days -1 and 0 (before the first caerulein injection), mice were intraperitoneally injected with anti-Ly6G antibody (clone 1A8, 25 µg per injection and mouse in PBS) or isotype-matched control. Neutrophil count in blood was performed 12 hours after the last caerulein injection. Each dot represents a single mouse (n=5-7). Mean±SEM of a representative experiment (N=2). Significance was assessed by unpaired Student's *t*-test between treatments; ** $p<0.01$; *** $p<0.001$.

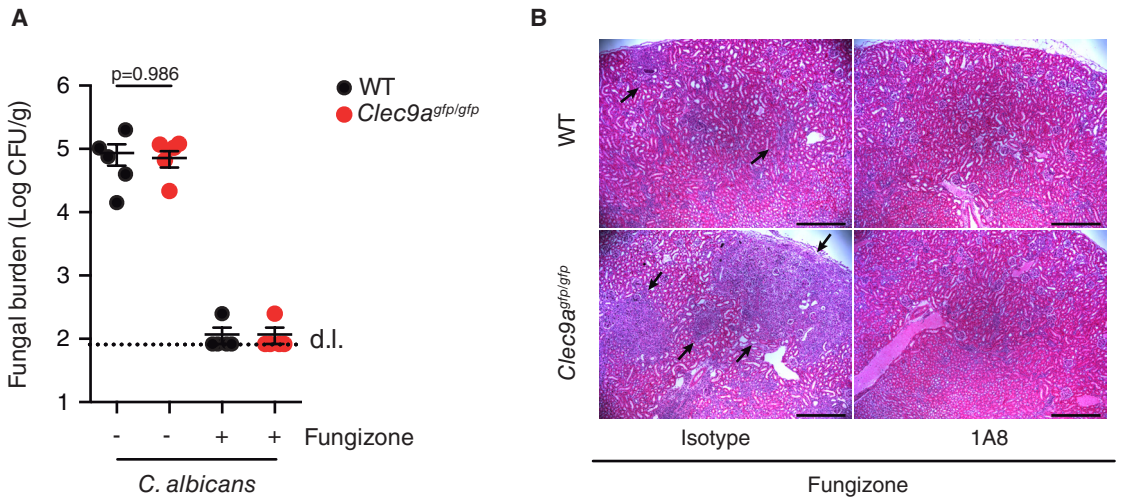

Fig. S6. Effective fungizone-induced elimination of fungal colonization and 1A8-mediated neutrophil depletion in kidney under these conditions during systemic candidiasis.

WT and *Clec9a*$^{gfp/gfp}$ mice were intravenously infected with 10^5 *Candida albicans*. (**A**) At day 3 post-infection, fungizone (+) (2 mg/kg body weight in PBS) or PBS (-) was intraperitoneally administered daily. After 3 days of treatment, renal fungal burden was determined as Colony Forming Units (CFU) per gram of kidney. d.l.: detection limit. (**B**) At day 3 post-infection, fungizone was administered together with anti-Ly6G antibody (clone 1A8, 25 µg per injection and mouse in PBS) or isotype-matched control. After 3 days of treatment, kidneys were collected and H&E staining was performed on renal sections. Arrows indicate neutrophil accumulation. Bar length=200 µm. (A) Each dot represents a single mouse. Mean±SEM of a representative experiment (N=3). Significance was assessed by unpaired Student's *t*-test between genotypes. (B) Representative images of n≥7 kidneys per condition (N=2).

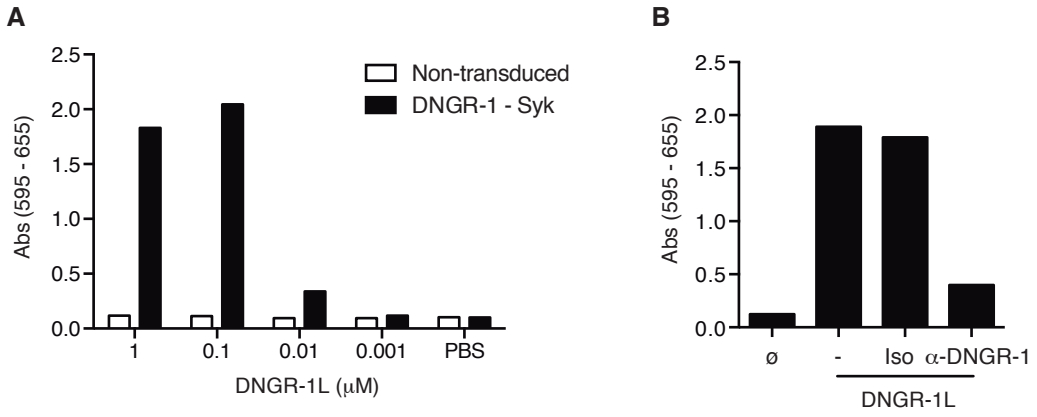

Fig. S7. DNGR-1 ligand efficiently signals through DNGR-1.

(**A**) B3Z NFAT reporter cells either transduced (DNGR-1–Syk) or not (Non-transduced) with the mouse version of *Clec9a* and *Syk*, were exposed to plated F-actin-based DNGR-1 ligand (DNGR-1L) at the indicated concentrations or to PBS. (**B**) DNGR-1–Syk B3Z NFAT reporter cells were pre-treated with culture medium (-), isotype control (Iso) or a blocking α-DNGR-1 antibody. After 30 minutes, they were exposed to 0.1 μM of DNGR-1L or left untreated (ø). (A, B) NFAT reporter activity was revealed by CPRG assay. A representative experiment of a single in vitro culture is shown (N=2).

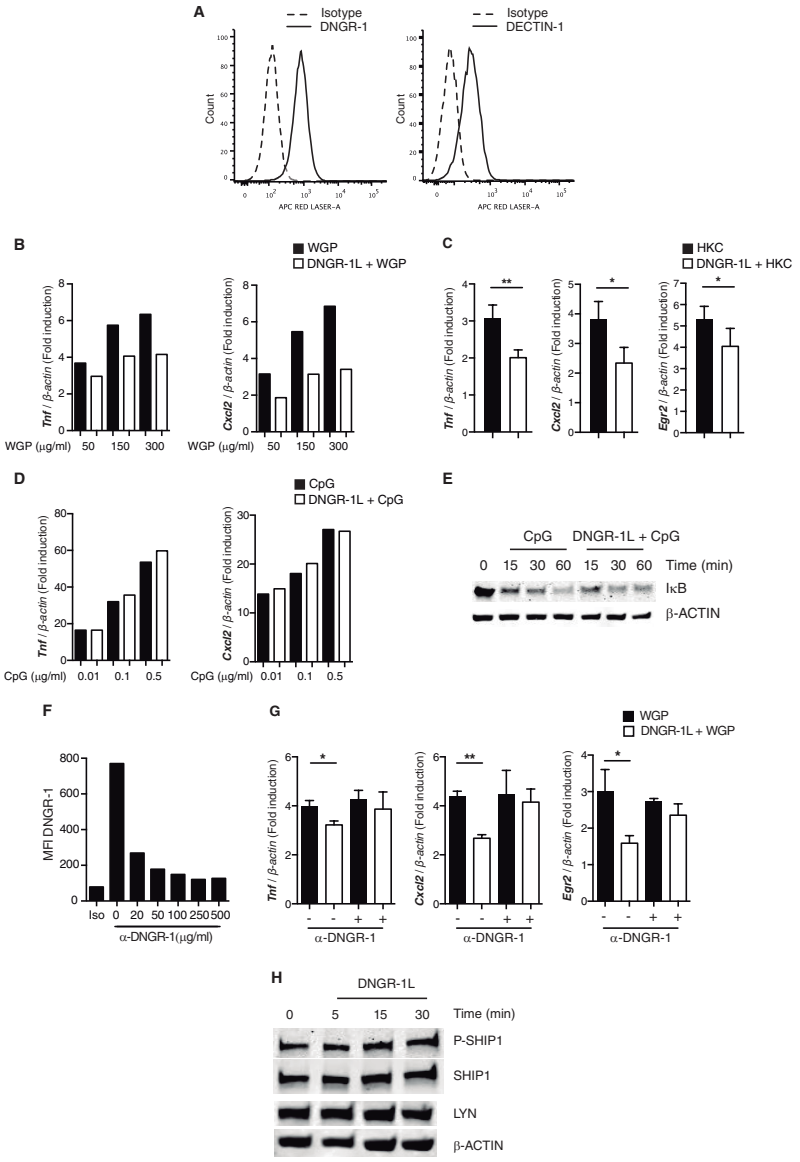

Fig. S8. DNGR-1 ligand triggers specific regulatory responses.

(**A**) DNGR-1 (left) and DECTIN-1 (right) surface expression was assessed on MutuDC cells by flow cytometry. MutuDCs cultured untreated or exposed to F-actin-myosin II (DNGR-1L), were stimulated with the indicated concentrations of WGP (**B**), heat-killed *Candida albicans* (HKC) (10:1 ratio) (**C**) or CpG (**D**) at the indicated concentrations for 4 hours. *Tnf*, *Cxcl2* and *Egr2* expression was analyzed by Q-PCR and expressed as fold induction versus non-stimulated cells. (**E**) MutuDCs were exposed or not to DNGR-1L and further stimulated with CpG for the given times. IkB degradation was analyzed by immunoblot. (**F**) MutuDCs were pre-treated for 30 minutes with 500 μg/ml of isotype control (Iso) or the indicated concentrations of a α-DNGR-1 blocking antibody. DNGR-1 surface expression was analyzed afterwards by flow cytometry. Remaining staining is shown as Mean Fluorescence Intensity (MFI). (**G**) MutuDCs were pre-incubated with isotype control (-) or blocking anti-DNGR-1 (+). Then, cells were exposed or not to DNGR-1L and further stimulated with WGP. *Tnf*, *Cxcl2* and *Egr2* expression was analyzed by Q-PCR. (**H**) MutuDCs were exposed to DNGR-1L for the given times. C-type lectin receptors-related regulatory mechanisms such as SHIP-1 phosphorylation and LYN degradation were assessed by immunoblot. (A,B,D-F,H) a representative experiment is shown of at least two performed. (C,G) Mean+SEM of pooled experiments including ≥3 individual cultures. Significance was assessed by paired Student's *t*-test between DNGR-1L-treated or not; **p* < 0.05; ***p* < 0.01.

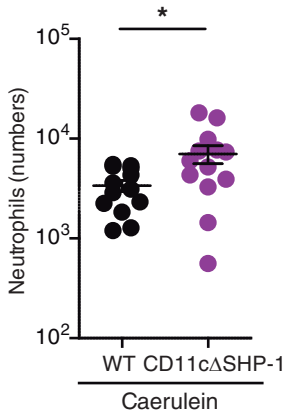

Fig. S9. Increased neutrophil infiltration in pancreas of CD11cΔSHP-1 mice during acute pancreatitis.

Acute pancreatitis was induced by seven intraperitonal injections of caerulein (50 micrograms/Kg) hourly for 6 hours in *Itgax*$^{+/cre}$*Ptpn6*$^{fl/fl}$ (CD11cΔSHP-1) and WT littermates mice. Animales were sacrificed 12 hours later and infiltrating neutrophils into the pancreas were monitored by flow cytometry. Mean±SEM of 2 pooled experiments. Each dot represents single mice. Significance was assessed by unpaired Student's *t*-test between genotypes; **p*< 0.05.

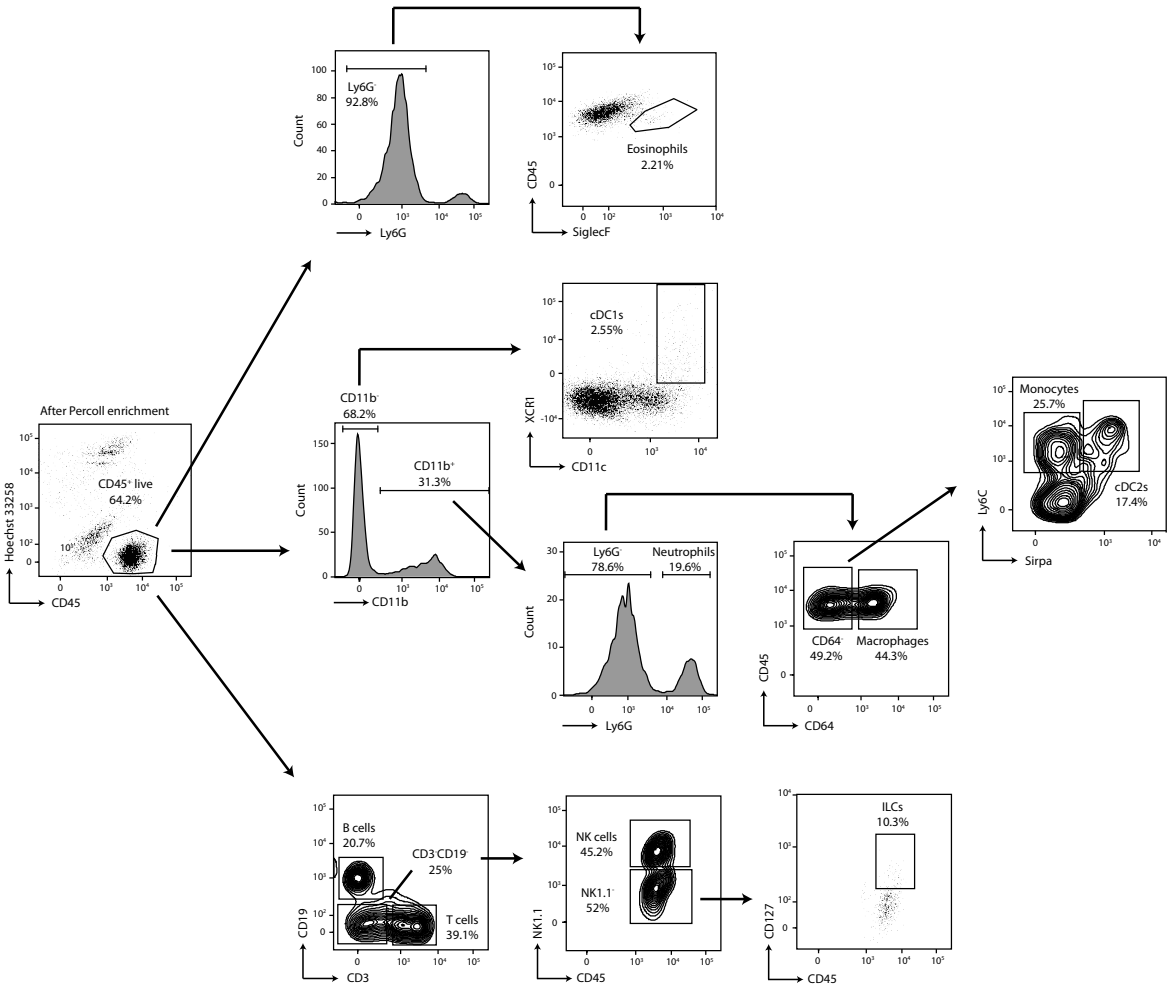

Fig. S10. Gating strategy used to sort infiltrating immune populations from *Candida albicans*-infected kidneys.

Candida albicans was intravenously injected in WT and *Clec9a*$^{gfp/gfp}$ mice. After 2.5 days, kidneys were collected, enriched in CD45^{+} cells by Percoll gradient centrifugation and the illustrated strategy was followed to FACS-sort (from top to bottom) eosinophils, type 1 cDCs (cDC1s), neutrophils, macrophages, monocytes, type 2 DCs (cDC2s), B cells, T cells, natural killer (NK) cells and innate lymphoid cells (ILCs). This gating strategy was used to determine the relative frequency of each population and to isolate each of them for further analysis of Mip-2 expression by qPCR.

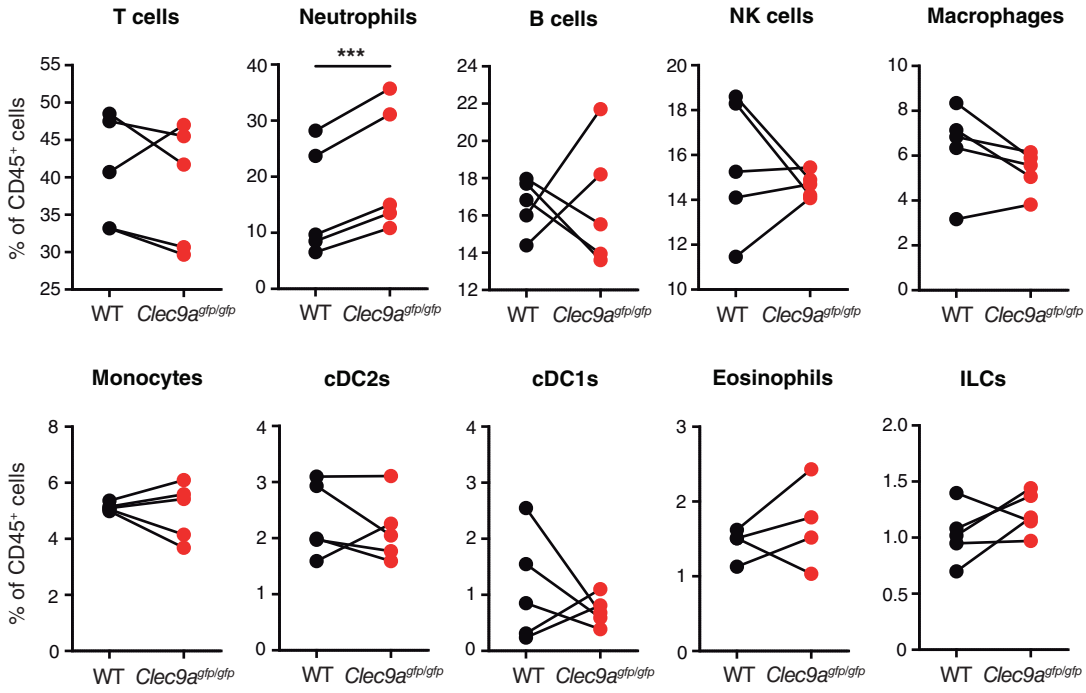

Fig. S11. Among kidney infiltrating CD45$^+$ immune cells during systemic candidiasis, only neutrophils are increased in DNGR-1-deficient mice.

Candida albicans was intravenously injected in WT and *Clec9a*$^{gfp/gfp}$ mice**.** After 2.5 days, kidneys were collected and enriched in CD45$^+$ cells by Percoll gradient centrifugation. The frequency of the indicated populations inside the CD45$^+$ compartment was analyzed. Paired analysis of 4 or 5 independent experiments represented by dots. Each dot includes a pool of ≥4 mice. Significance was assessed by paired Student's *t*-test between genotypes; ***p< 0.001

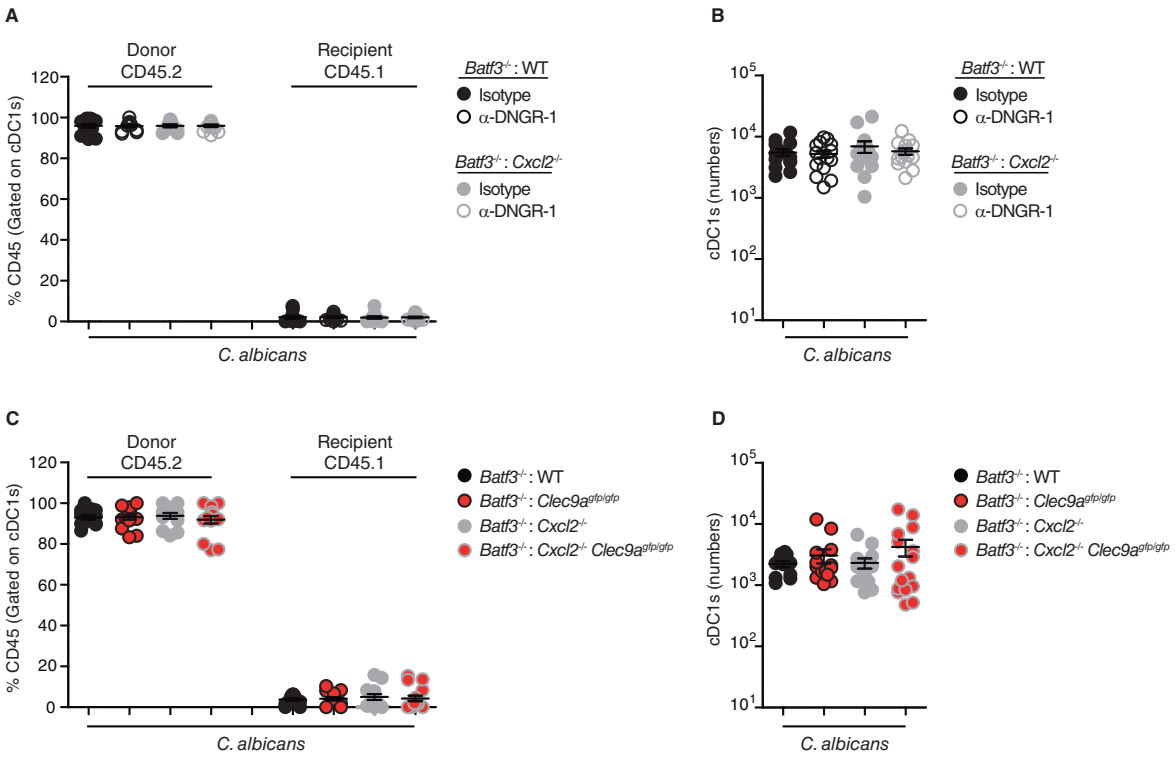

Fig. S12. Reconstitution of mixed bone marrow chimeric mice is not affected by the genotype of the transferred cells.

Mixed bone marrow (BM) chimeric mice were generated as follows. (**A**, **B**) Lethally irradiated B6/SJL CD45.1 recipient WT mice were transferred with a mix of 50% of *Batf3*$^{-/-}$ BM cells (CD45.2) and 50% of WT or *Cxcl2*$^{-/-}$ BM cells (CD45.2). Mice were infected with *C. albicans* and anti-DNGR-1 or isotype control antibodies were intraperitoneally injected on day -1 and daily after infection. After 6 days of infection, the expression of the CD45.1 or CD45.2 allotype (A) and the number of renal cDC1s (B) was determined by flow cytometry. (**C**, **D**) Alternatively, mixed bone marrow chimeras were generated as described above, consisting of 50% of *Batf3*$^{-/-}$ BM cells and 50% of either WT, *Clec9a*$^{gfp/gfp}$, *Cxcl2*$^{-/-}$ or *Cxcl2*$^{-/-}$*Clec9a*$^{gfp/gfp}$ (CD45.2) BM cells. Mice were infected with *C. albicans* and, after 6 days of infection, the expression of the CD45.1 or CD45.2 allotype (C) and the number of renal cDC1s (D) was determined by flow cytometry. (A-D) Mean±SEM of 2 pooled experiments. Each dot represents single mice (n=11-15).

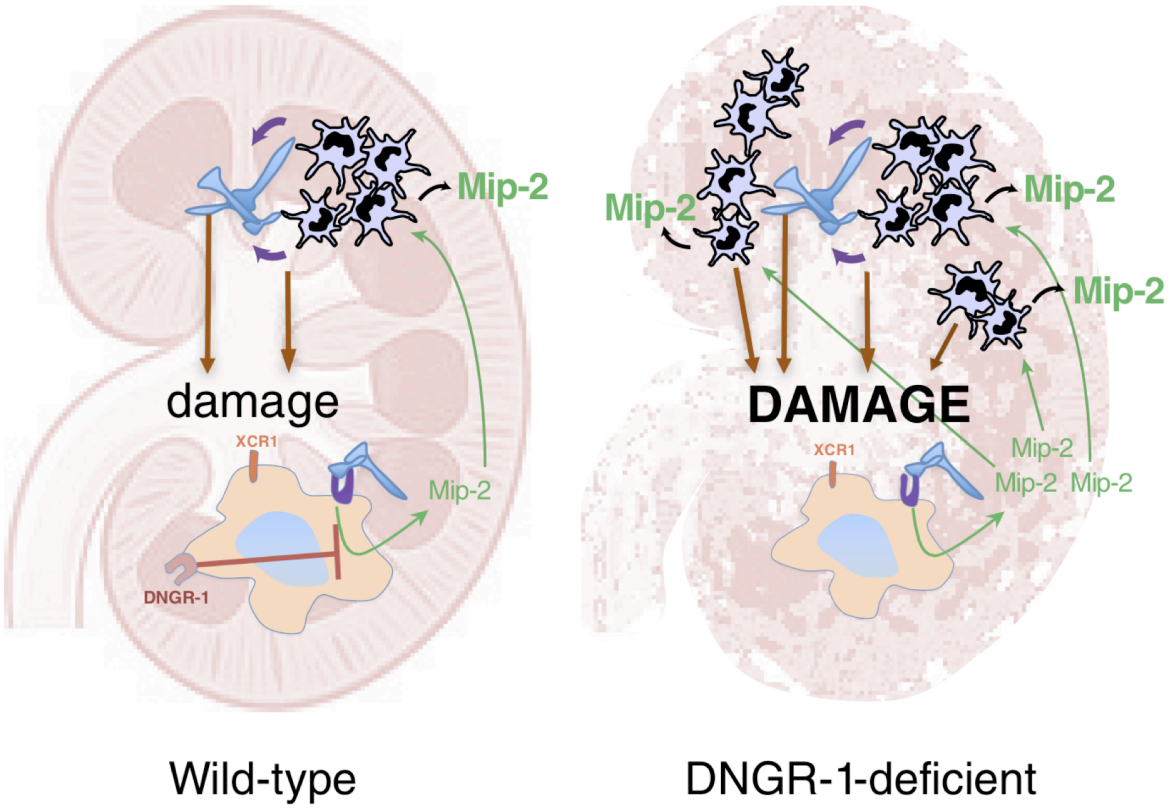

Fig. S13. Working model of the DNGR-1-mediated tissue damage control mechanism under systemic *Candida albicans* infection.

In wild-type mice, sensing of tissue damage by DNGR-1 expressing-cDC1s dampens Mip-2 production by these cells, keeping the infiltration of potentially harmful neutrophils in check. However, in the presence of ongoing tissue damage that is not properly sensed in DNGR-1-deficient mice, the higher production of Mip-2 by cDC1s ignites increased entry of neutrophils that mediate immunopathology.