Supporting Information

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SI Materials and Methods

Mice. Female wild-type (WT) C57BL/6 mice were purchased from Harlan-Winkelmann. OT I mice, RAG1$^{-/-}$ (1), RAG2$^{-/-}$ C57BL/6 (2), and RAG2$^{-/-}\gamma c^{-/-}$ (3) mice were bred at the animal facility of the Helmholtz Centre for Infection Research. Mb1-cre mice have been described (4). FcR$\gamma^{-/-}$ (5), FcγRII$^{-/-}$ (6), and FcγRI/II/III$^{-/-}$ mice were provided by J. Sjef Verbeek (Leiden University Medical Center, Leiden, The Netherlands). C3$^{-/-}$ mice (7) were provided by Andreas Klos (Hannover Medical School). MR$^{-/-}$ mice (8) were provided by Christian Kurts (Bonn University, Bonn, Germany). Mice were used between 8–12 wk of age. All mice were bred and maintained in specific pathogen-free conditions.

Isolation and Culture of Splenocytes, T Cells, and Bone Marrow Cells.
Splenocytes. Spleen cells were prepared by gentle flushing out the spleens with complete IMDM. Erythrocytes were lysed for 2 min in ACK lysis buffer (0.15 M NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA) and washed two times in PBS. Cell clumps were removed by passage through a 50-μm nylon filter. Splenocytes preparation was carried out strictly on ice. Cells were further used for staining with different Abs, flow cytometry analysis, or cell sorting.
T cells. OT I ($OVA_{257–264}$-specific CD8$^+$ T cells) cells were isolated from lymph nodes (s.c. and mesenteric) and spleen. Single-cell suspension was purified by using the CD8 negative isolation kits (Dynal) containing Abs against B220, CD11b, Ter-119, CD16/32, and CD4 by following the protocol provided by the manufacturer. Cell preparations contained more than 90% of the desired cell population and were essentially free of CD11c^{hi} cells as determined by flow cytometry using Abs specific for CD8 and CD11c. For antigen presentation assays, OT I cells were stained with 1 μM CFSE (Molecular Probes) for 10 min at 37 °C according to the manufacturer's protocol.
BMDCs. Bone marrow cells were cultured in complete RPMI medium 1640 containing 10% (vol/vol) FCS and mouse granulocyte macrophage-colony stimulating factor (20 ng/mL; Peprotech) for 10 d. On day 9, BMDCs were stimulated with LPS (0.1 μg/mL) for 18 h.

Flow Cytometry and Cell Sorting. Single-cell suspensions were treated with anti-mouse CD16/CD32 BD Fc Block (2.4G2; Becton Dickinson) for 10 min followed by staining with appropriate mAbs for 20 min on ice. Abs used in this work included anti-mouse CD11c (clone N418) conjugated with allophycocyanin (APC) or phycoerythrin-Cy7 (PE-Cy7), CD11b (M1/70) PE-Cy7, CD8α (53-6.7) PacificBlue, fluorescein isothiocyanate (FITC) or phycoerythrin (PE), B220 (RA3-6B2) APC-Alexa Fluor 750, CD4 (GK1.5) PE, CD86 (GL1) FITC, CD40 (HM40-3) FITC, CD80 (16-10A1) PE, CD1d (1B1) PE, and ICAM-1 (YN1/1.7.4) PE, all purchased from eBioscience. H-2K^b (Y-3) FITC and I-A^b (M5/114.15.2) FITC were purified and conjugated with FITC in our laboratory. Anti-mouse CD14 (Sa14-2) APC and CD64 (FcγRI) (X54-5/7.1) PE were purchased from BioLegend. For analysis of surface expression of C-type lectin receptors, the Abs used were as follows: anti-mouse dectin-1 (218820) APC (R&D Systems), CD209b (SIGN-R1) (22D1) APC (eBioscience), CD209a (DC-SIGN) (MMD3) eFluor-660 (eBioscience), and CD206 (MR) (C068C2) PE (BioLegend). Flow cytometric analysis and sorting were performed by using FACSCanto, LSRII, and FACSAria (Becton Dickinson). All samples during the sorting procedure were kept at 4 °C. The data were analyzed by using FACSDiva, version 6.1.1 software (Becton Dickinson) and FlowJo, version 9.4.8 (Tree Star).

Analysis of Antigen Presentation. IgG-OVA immunocomplexes were prepared by incubation of OVA (Profos) with polyclonal rabbit anti-OVA IgG (ICN Biomedicals) for 30 min at 37 °C in a mass ratio 1:50. DCs were loaded with 10× concentrated IgG-OVA for 1 h at 37 °C, further washed intensively. To prepare cell-associated antigen, EG7-OVA and EL4 cells were harvested from culture bottles and counted. Further 1×10^6 cells were UV irradiated, using an UV Crosslinker, with 9 mJ/cm^2. Next, cells were washed intensively with PBS and plated (1×10^4 per well) on 96-well plates together with splenic cDCs (1×10^4 per well). Subsequently, the cells were washed three times and resuspended in complete IMDM containing 2×10^5 CFSE-labeled OT I cells. For inhibition of endosomal acidification, chloroquine (InvivoGen) was titrated into DC–T-cell cocultures.

Determination of Antigen Uptake and Processing. Sorted cDCs were incubated with 62.5 μg/mL DQ-OVA (conjugated with BODIPY FL; Molecular Probes) for 45 min at 37 °C or on ice. Further, cDCs were washed carefully and analyzed by FACS. For analysis of uptake of OVA-Cy5, soluble OVA was conjugated to Cy5. The labeling procedure involved gel filtration as a final step for removal of low molecular mass molecules such as unbound fluorochrome. The concentration of OVA-Cy5 was determined by measurement of OD_{280}. Sorted DCs were incubated with indicated concentrations of OVA-Cy5 for 1 h, than washed carefully. Uptake of fluorescent OVA was determined by FACS.

Microarrays. RNA isolation, cDNA preparation, and DNA microarray analysis of gene expression was performed at the gene array facility of the Helmholtz Centre for Infection Research in Braunschweig. Fluorescent images of hybridized microarrays (Affymetrix; MOE-430 version 2.0) were obtained by using an Affymetrix Genechip Scanner. Microarray data were analyzed by using BioConductor Suite 2.1 software. All samples were repeated two times with individually sorted cells and averaged.

Reconstitution of RAG-Deficient Mice with T Cells. WT and RAG-deficient mice were injected i.v. with CD3$^+$CD4$^+$ and CD3$^+$CD8$^+$ splenic T cells (3×10^6 per mouse). Twenty-one days after reconstitution, splenic cDCs were sorted from recipient mice and used as APCs in OT I antigen presentation assay.

In Vivo i.v. Administration of Serum, Immunoglobulins, and Mannan.
Serum. WT and RAG$^{-/-}$ mice were i.v. injected with 100 μL of serum isolated from blood of WT or RAG-deficient mice. Injection was repeated once per week for 3 wk to allow complete turnover of the DC pool in the spleen. Further, recipient mice were killed, and splenic cDCs were sorted and tested for their ability to cross-present OVA.
Immunoglobulins. WT and RAG$^{-/-}$ mice were i.v. injected with 7.5 μg of Functional Grade Purified IgG1, κ (clone P3.6.2.8.1; eBioscience), 7.5 μg of Functional Grade Purified IgM (clone 11E10, reactive with LPS; eBioscience), IgM + IgG (7.5 μg + 7.5 μg), or PBS on day 0 and day 7. After 21 d, mice were killed and splenic cDCs were sorted. The cells were loaded with soluble OVA and used as APCs in OT I assays.
Mannan. WT and RAG$^{-/-}$ mice were i.v. injected with 100 μL (2 mg/mL) of mannan from *Saccharomyces cerevisiae* (Sigma Aldrich), and 4 h later, some groups were additionally injected i.v. with Ig as described above. Mannan was injected twice per week within 3 wk. Next, mice were killed and sorted splenic cDCs were tested in cross-presentation assay.

Preparation of Fab Fragments from Rat IgG. Purified rat anti-mouse CD16/32 IgG2b (clone FCR-4G8, in-house preparation) was digested for 16 h at 37°C with immobilized papain (Pierce) in papain digestion buffer (20 mM NaPi at pH 7.2, 10 mM EDTA at pH 8.0, and 20 mM Cysteine). The digest was purified by FPLC on HiTrap DEAE Sepharose fast flow column (GE Healthcare). The purity of preparation was validated by SDS/PAGE.

Binding of Immunoglobulins to CLRs. WT and MR-deficient BMDCs were counted, washed twice with PBS, and incubated for 15 min at 37 °C after each wash. Further cells were incubated with Fab fragments generated from anti-CD16/32 rat IgG. Next, cells (5×10^5 per sample) were incubated on ice with mouse IgG labeled with PE (P3.6.2.8.1; eBioscience) for 1 h. Cells were analyzed by flow cytometry.

Western Blot. Splenic cDCs from WT and RAG-deficient mice were sorted as described in "Flow Cytometry and Cell Sorting Section." Cells (1×10^6) were lysed in 100 μL of sample buffer and boiled for 10 min at 96 °C. Next, 15 μL of protein extracts was subjected to electrophoresis in 10% SDS/PAGE gels, transferred to a nitrocellulose membrane, and blocked with 0.1% Tween 20/5% (wt/vol) of milk TBS. The nitrocellulose membrane was then incubated overnight with primary Abs (goat anti-mouse Cathepsin B, Cathepsin D, and Cathepsin E; all from R&D Systems), washed, and incubated with secondary antibody donkey anti-goat conjugated to HRP. The membrane was subsequently developed by using the ECL Western Blotting Detection Kit (Amersham Biosciences). Quantification of bands intensity was performed with ImageJ and GraphPad Prism softwares.

Fluorescence Microscopy. Splenic cDCs were sorted from WT and RAG-deficient mice as described above. Further, cells were plated at 5×10^5 per well on a glass slides in a 24-well plate and incubated overnight at 37 °C. Next day, cDCs were loaded with 62.5 μg/mL DQ-OVA (Invitrogen) for 40 min at 37 °C in complete IMDM. After three washes, cells were further incubated for 2 h at 37 °C, washed in PBS, and fixed in 3% (vol/vol) paraformaldehyde at room temperature for 15 min. The antibodies used were as follows: rat anti-LAMP-2 (Hybridoma Bank), mouse anti-EEA-1 (BD Biosciences), and anti-mouse and anti-rat Alexa546 conjugated (Molecular Probes). Cathepsins B, D, and E were detected by using biotinylated Abs (all from R&D Systems).

Fixed cells on coverslips were quenched for 15 min in 50 mM Glycin in PBS followed by 30-min incubation with 1% (wt/vol) BSA (Sigma) and 0.01% Saponin (Sigma) in PBS. The primary and secondary antibodies were diluted in PBS and incubated for 1 h. Nuclear staining was performed by using 4',6-diamidino-2-phenylindole. After staining, coverslips were mounted on slides by using aqueous mounting medium (Dako Cytomation). Samples were analyzed by confocal fluorescence microscopy using a Leica SP5 microscope (Leica Microsystems).

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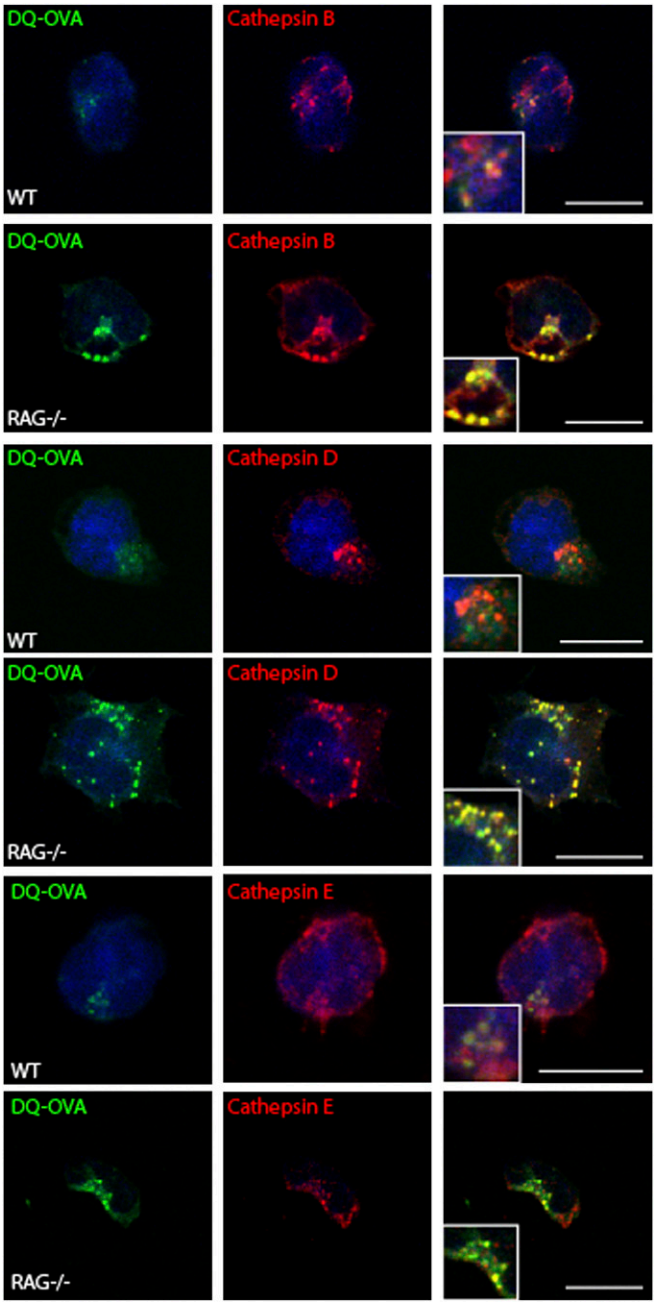

Fig. S1. Cathepsins colocalize with OVA degradation product in splenic cDCs isolated from RAG-deficient mice. WT and RAG$^{-/-}$ cDCs were loaded with 62.5μg/mL DQ-OVA for 45 min, washed extensively, and incubated at 37 °C for 2 h. Next, cells were fixed and stained for Catehpsins B, D, or E. Pictures are representative of multiple cells (sorted from 20 animals per group). DAPI was used as nuclear staining. (Scale bars: 10 μm.)

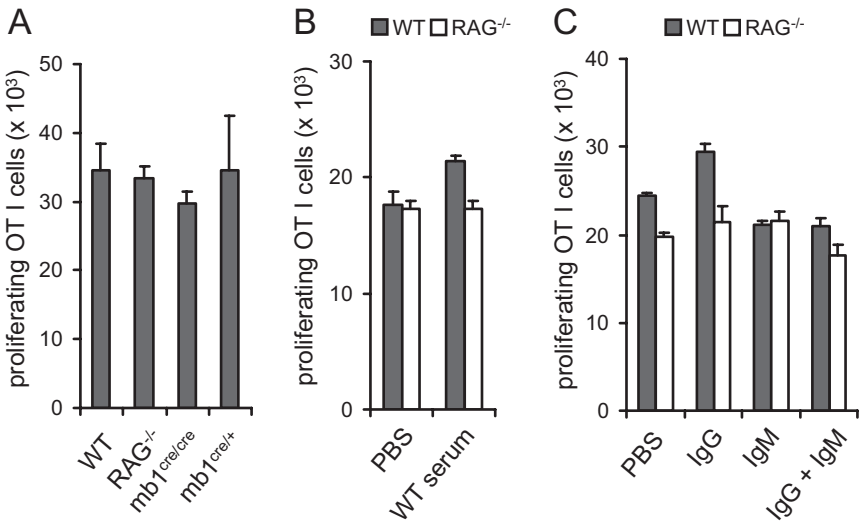

Fig. S2. Presentation of $OVA_{257–264}$ peptide is not altered in splenic cDCs. (*A*) Splenic cDCs from WT, $RAG^{-/-}$, $mb1^{cre/cre}$, and $mb1^{cre/+}$ mice were loaded with indicated concentrations of SIINFEKL ($OVA_{257–264}$) peptide and incubated with CFSE-labeled OT I cells for 1.5 d. (*B*) WT and $RAG^{-/-}$ mice were injected three times within 21 d i.v. with serum collected from WT and $RAG^{-/-}$ mice. DCs were sorted and loaded with indicated concentrations of SIINFEKL ($OVA_{257–264}$) peptide, incubated with CFSE-labeled OT I cells for 1.5 d. (*C*) WT and $RAG^{-/-}$ mice were injected twice within 21 d i.v. with murine IgG (7.5 μg per mouse) and/or murine IgM (7.5 μg per mouse). Next, cDCs were sorted and loaded with indicated concentrations of SIINFEKL ($OVA_{257–264}$) peptide, incubated with CFSE-labeled OT I cells for 1.5 d. The proliferative response of T cells was enumerated by flow cytometry. Numbers of proliferating cells are shown (mean + SEM). Data are representative of two or three independent experiments with minimum three mice per group.

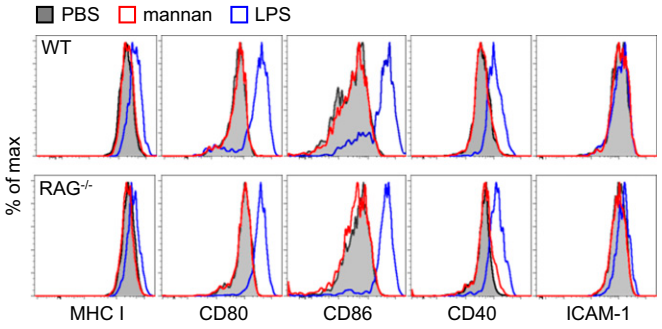

Fig. S3. Injection of mannan from *S. cerevisiae* does not induce maturation of splenic cDCs. WT and RAG-deficient mice were injected i.v. with PBS, mannan (200 μg per mouse), and LPS (50 μg per mouse). Sixteen hours later, splenic cDCs were stained with monoclonal Ab against surface antigens indicated on the *x* axis of the histograms. Representative data are shown.

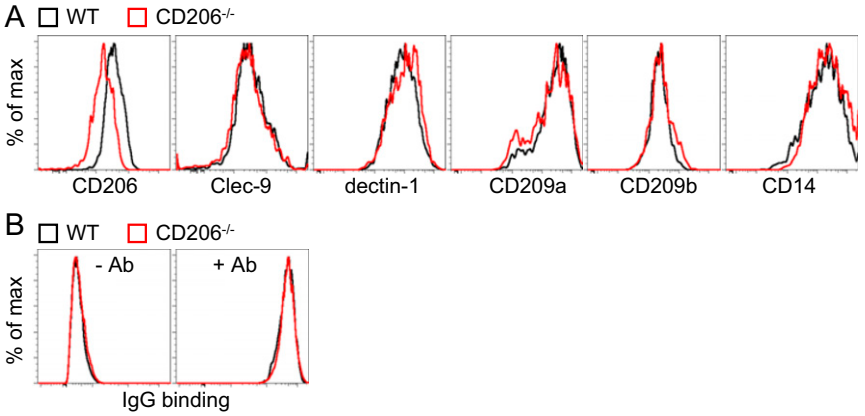

Fig. S4. MR alone is not responsible for Ig binding. (*A*) BMDCs from WT and MR-deficient mice after LPS-induced maturation were analyzed for surface expression of indicated CLRs. (*B*) BMDCs from WT and MR-deficient mice were incubated with Fab fragments generated from rat anti-mouse CD16/32 IgG and further tested for their ability to bind mouse IgG conjugated with PE. Representative data are shown.