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Supplementary Materials for

CD169$^+$ macrophages orchestrate innate immune responses by regulating bacterial localization in the spleen

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Other Supplementary Material for this manuscript includes the following:
(available at immunology.sciencemag.org/cgi/content/full/2/16/eaah5520/DC1)

Movie S1 (.mov format). *Listeria* is captured by $CD169^+$ macrophages early after infection.
Movie S2 (.mov format). *Listeria* is captured by $CD169^+$ macrophages early after infection.
Movie S3 (.mov format). $CD169^+$ macrophages transfer bacteria to $CD8\alpha^+$ DCs.
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Materials and Methods

Cell sorting and purification for RNA sequencing: Spleen cells from naïve or infected (12 hrs post infection) C57BL/6J mice were isolated as described above. Leukocytes obtained were negatively enriched (depleted B220, CD3, NK1.1, and GR-1) using STEMCELL Technologies and negatively selected cells were pooled. Enriched fractions were stained for live-dead marker, CD11b, CD11c, MHCII, CD8α, CD169, F/480, B220, NK1.1**,** and GR-1 (Table S6). CD169+ and F4/80+ cells were sorted on a Becton-Dickinson FacsAria II. RNAeasy Plus Microkit (Qiagen) was used to extract total RNA from sorted cells. RNA integrity was validated using an Agilent 2100 Bioanalyzer, and only samples with RIN values ≥ 7 were used (Fig. S3B)

RNA-sequencing and data-set analysis: RNA-Sequencing libraries were prepared using the Illumina TruSeq Stranded mRNA library kit at the Center for Genome Innovation at UCONN Storrs, CT. The individual libraries were pooled together and run over 2 lanes on the Illumina HiSeq2500. Trimmomatic v0.32 was used to remove adapter sequences and bases with base quality lower than three and trimmed reads were mapped to reference genome (GRCm38.p84) with TopHat (v.2.0.13). G option on Cufflinks (v.2.2.1) was used for transcript assembly and FPKM quantification. Data was normalized using log2 transformation with 1 was added to the raw signal (FPKM+1) followed by quantile normalization using the preprocess Core package in R. From a total of 47,443 genes, 30,107 were excluded and only 17,336 differentially expressed genes were used for statistical analysis. To ensure reproducibility between biological replicates, Pearson correlation coefficients were calculated on the $\log_2$(FPKM+1) values and coefficient values ranged from 0.98-0.9 (Fig. S3C). Similarly, genes showed clear separation between CD169+ macrophages from naïve and infected mice as well as concordance among group

replicates. Statistical analysis was performed using fold change and Independent T-test per comparison pair. The up-regulated genes were filtered using FC≥2 and FDR<0.05. Down regulated genes were filtered using FC≤-2 and FDR<0.05. These differentially expressed genes are graphically depicted in heatmaps with hierarchical clustering using z-scores. For the identified up-and down-regulated differentially expressed genes in each comparison group, we performed a gene ontology (GO) enrichment analysis using geneXplain bioinformatics software platform (v. 2017.1) and its PROTEOME database. The top fifteen GO terms, according to p-values, were reported for different comparison groups and with a threshold for p-value $\leq 10^{-4}$. The sets of up- and down-regulated genes identified in each comparison were subjected to the promoter analysis using TRANSFAC (*36*). Each promoter was defined as the sequence within −500 to +100 coordinates, where the TSS of the main transcript of each gene was the point 0. The TFBS search on promoter sequences was done using the MATCH algorithm (*37, 38*) integrated in the GeneXplain platform and executed within the pre-defined workflows. In each comparison group, we used an enrichment fold (FE) ≥1.5.

Bacteria quantification: Tissues were processed into single cell suspensions and were incubated at 4°C in 1% saponin for 1 hour. Tissue lysates were plated on brain heart infusion (BHI; Becton-Dickinson) agar containing 50 μg/ml streptomycin. Streaked BHI plates were incubated overnight at 37°C. Equal volumes of whole blood was collected and blood cells were lysed in 1% saponin. Whole blood lysates were plated on BHI agar and incubated overnight at 37°C. Facs Aria sorted cell populations were incubated at 4°C in 1 % saponin for 1 hour. Cell lysates were plated on BHI agar and incubated overnight at 37°C.

Flow cytometry: Spleens were incubated in RPMI containing 10% FCS and 100 U/mL collagenase IV for 30 minutes at 37°C. Subsequently the treated splenic tissue was dissociated into single cell suspension, treated with Tris-buffered ammonium chloride (TAC), and remaining leukocytes were stained for live-dead (Invitrogen, A-10168), CD45, CD11b, CD11c, CD169, CD8α, NK1.1, CD3,, MHCII, F4/80, Ly6C), Ly6G or CCR2 (Table S6). Cell suspensions were fixed and acquired using a Becton-Dickinson LSR-II. Cellular differentials were analyzed using FlowJo software.

Confocal microscopy: Spleen tissue was fixed overnight at 4°C in PFA, lysine, and periodate (PLP) buffer and dehydrated in 30% sucrose overnight prior to embedding in OCT. Cryopreserved spleen tissue was sectioned and stained in 1X phosphate-buffered saline (PBS) containing 2% FCS, 2% goat serum, 0.5% Fc block and 0.05% Tween-20. Staining buffer was supplemented with 0.3% Triton-X for intracellular cytokine stains. Spleen sections were stained for L.m., CD169, CD11c, CD11b, Ly6G, B220, CD8α, NK1.1 or IFN-γ (Table S6). Images were acquired on a Zeiss LSM 780 microscope using Zen software from Carl Zeiss.

Confocal imaging analysis: Confocal images were analyzed using Imaris x64 version 7.7.0 (Bitplane). Where noted, spot detection minus background was used to render bacteria and in-situ IFN-γ stain. CD8α+ DCs were identified using the Colocalization function of the Imaris software where CD11c and CD8α co-expression on cells was determined using a colocalization coefficient. Isosurface function of the ImarisXT was used to construct 3D isosurface of the macrophage cell volume using the fluorescence of CD169 surface stain and threshold was adjusted to separate object from background. To quantify bacteria that were inside or outside the

CD169+ macrophages spots of bacteria were color coded and masked to determine whether the bacteria were intracellular or extracellular of isosurfaced object.

Multiplex: Cytokine and chemokine protein was quantified from spleen lysate using Mouse Cytokine 23-plex Assay (Biorad).

Statistics: All statistical analyses were performed using Prism GraphPad Software. For experiments comparing 2 groups, two-tailed Student t-test was used to determine statistical significance; p values <0.05 were considered significant. For bacterial quantification experiments non-Gaussian distribution was assumed and Mann-Whitney test performed. For experiments comparing three or more groups one-way ANOVA, Bonferroni test was used to determine statistical significance for sample groups that assume Gaussian distribution or Kruskal-Wallis H test with sample groups that assume non-Gaussian distribution; p values <0.05 were considered significant. Data are represented as mean ± SEM, unless otherwise specified.

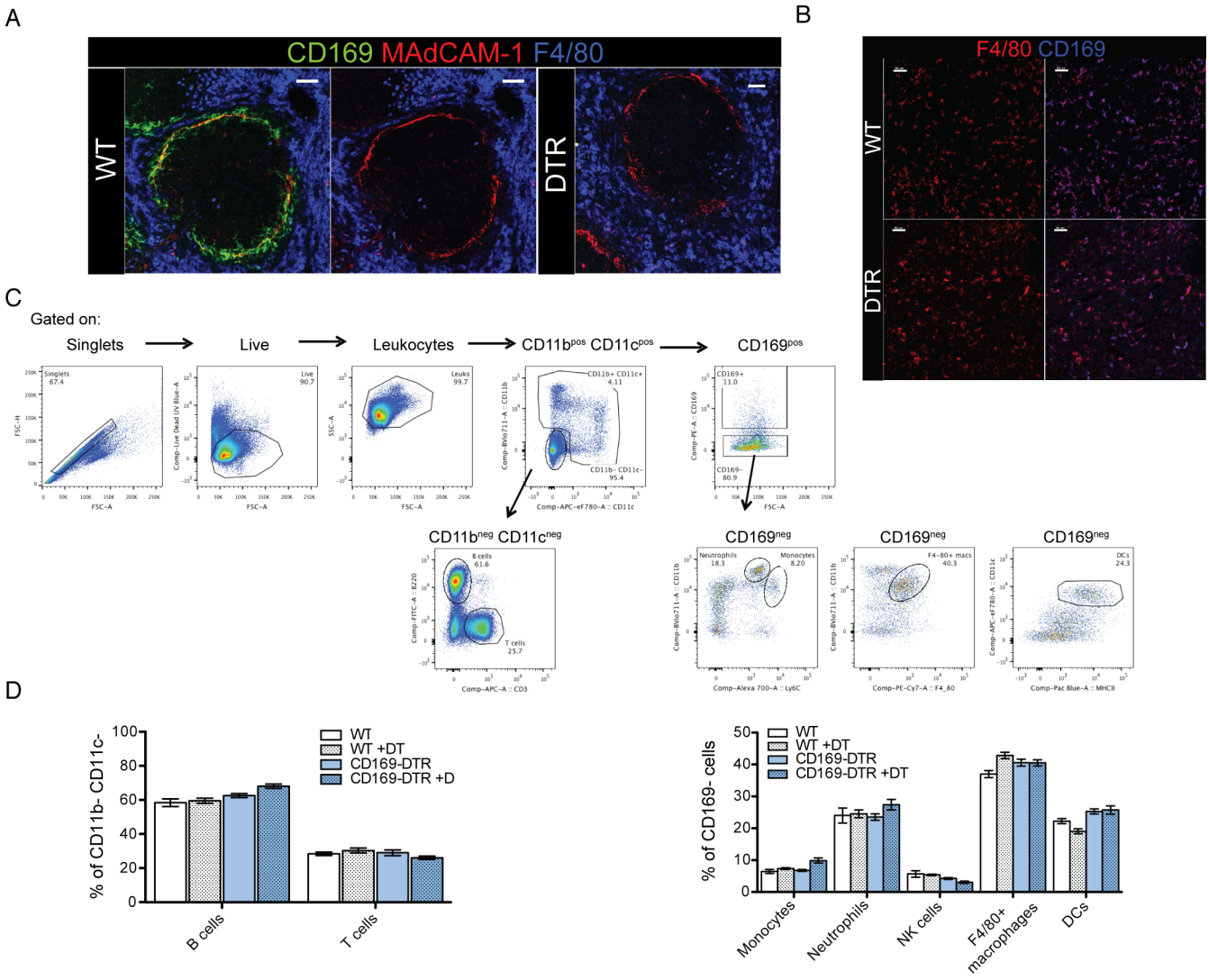


Fig. S1. Characterization of WT and CD169-DTR mice. (A) Representative confocal images of spleen sections depicting CD169+ macrophage depletion in CD169-DTR mice, but normal MadCAM-1+ cells in the marginal sinus. Scale bar, 50 μm. (B) Confocal images of liver sections from WT mice or CD169-DTR mice 24 hpi stained for F4/80 and CD169. Scale bar, 80 μm. (C) Representative FACS plot gating strategy of immune cell in naive spleen of WT mice. (D) Frequency of monocytes, neutrophils, NK cells, red pulp macrophages (F4/80+), DCs, B cells and T cells in spleens of naive WT mice or CD169-DTR mice untreated and 48 hours post diphtheria toxin treatment. Shown as mean ± SEM, n=4, representative of 3 separate experiments.

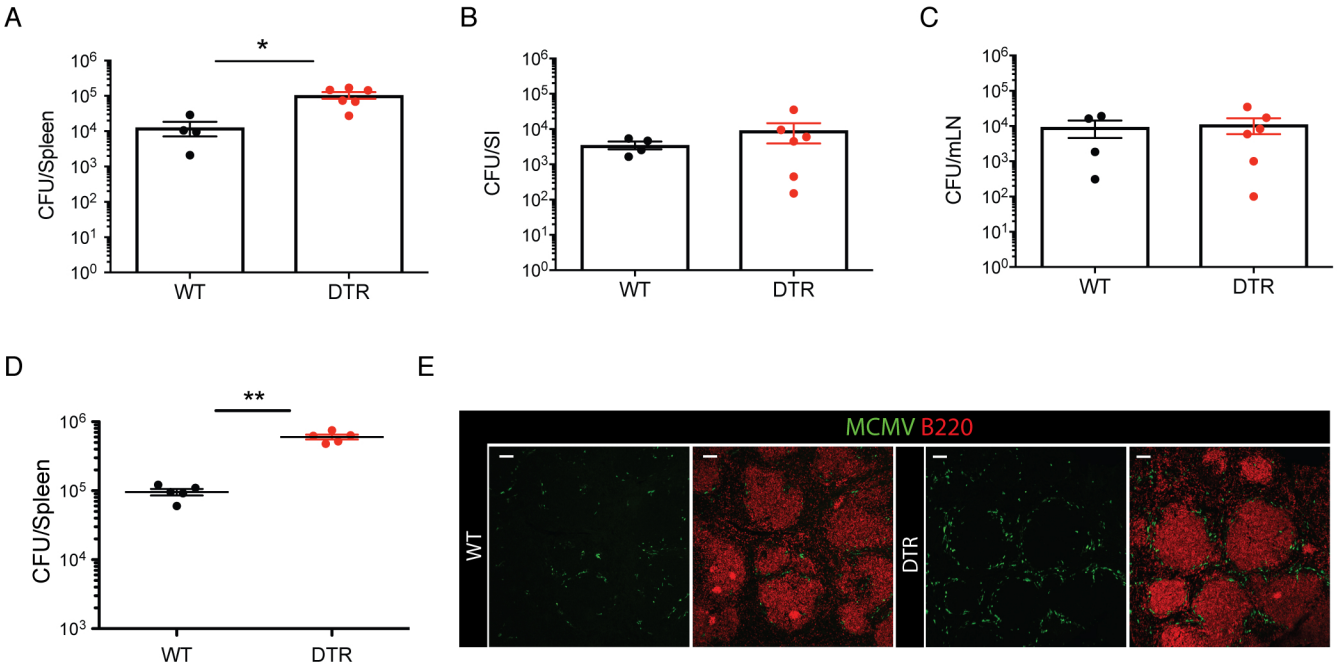


Fig. S2. Splenic CD169+ macrophages are essential for protection against various blood-borne pathogens. (A-C) L.m. quantification from (A) spleen (B) small intestine and (C) mesenteric LNs of WT or CD169-DTR mice 2 days post oral infection with L.m. Each symbol represents an individual mouse, shown as mean ± SEM (* $p<0.05$ Mann-Whitney). (D) E. coli quantification from spleens of WT mice or CD169-DTR mice at 24 hpi. Each symbol represents an individual mouse, shown as mean ± SEM (** $P<0.005$, Mann-Whitney). (E) Confocal images of WT mice or CD169-DTR mice 72 hpi with MCMV-GFP. Spleen sections were imaged for MCMV and B cells. Scale bar, 100 μm. All data are representative of 2 separate experiments with 3-6 mice per group.

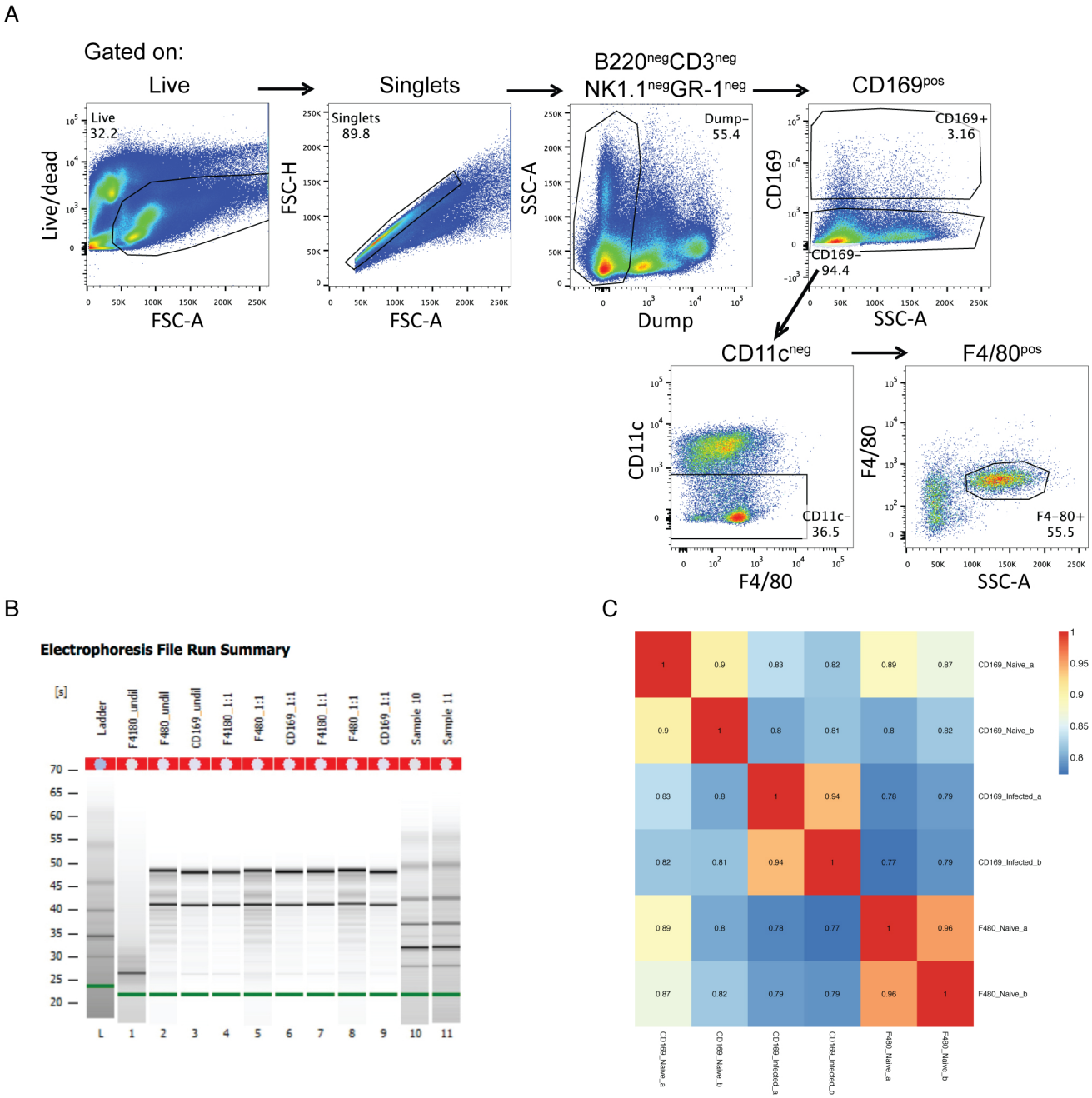


Fig. S3. Sorting strategy and quality control for RNA sequencing. (A) General gating strategy used to sort total CD169+ macrophages and F4/80+ red pulp macrophages from spleens prior to RNA sequencing. (B) Representative gel image of high sensitivity RNA ScreenTape used to determine RNA integrity. Image was scaled to sample and scaled to view larger molecular weight range (contrast 50%). (C) Correlation matrix of Pearson's coefficient of the RNA sequencing libraries. Values are based on the Log2(FPKM+1) of 17,336 genes that were used for downstream statistical analysis.

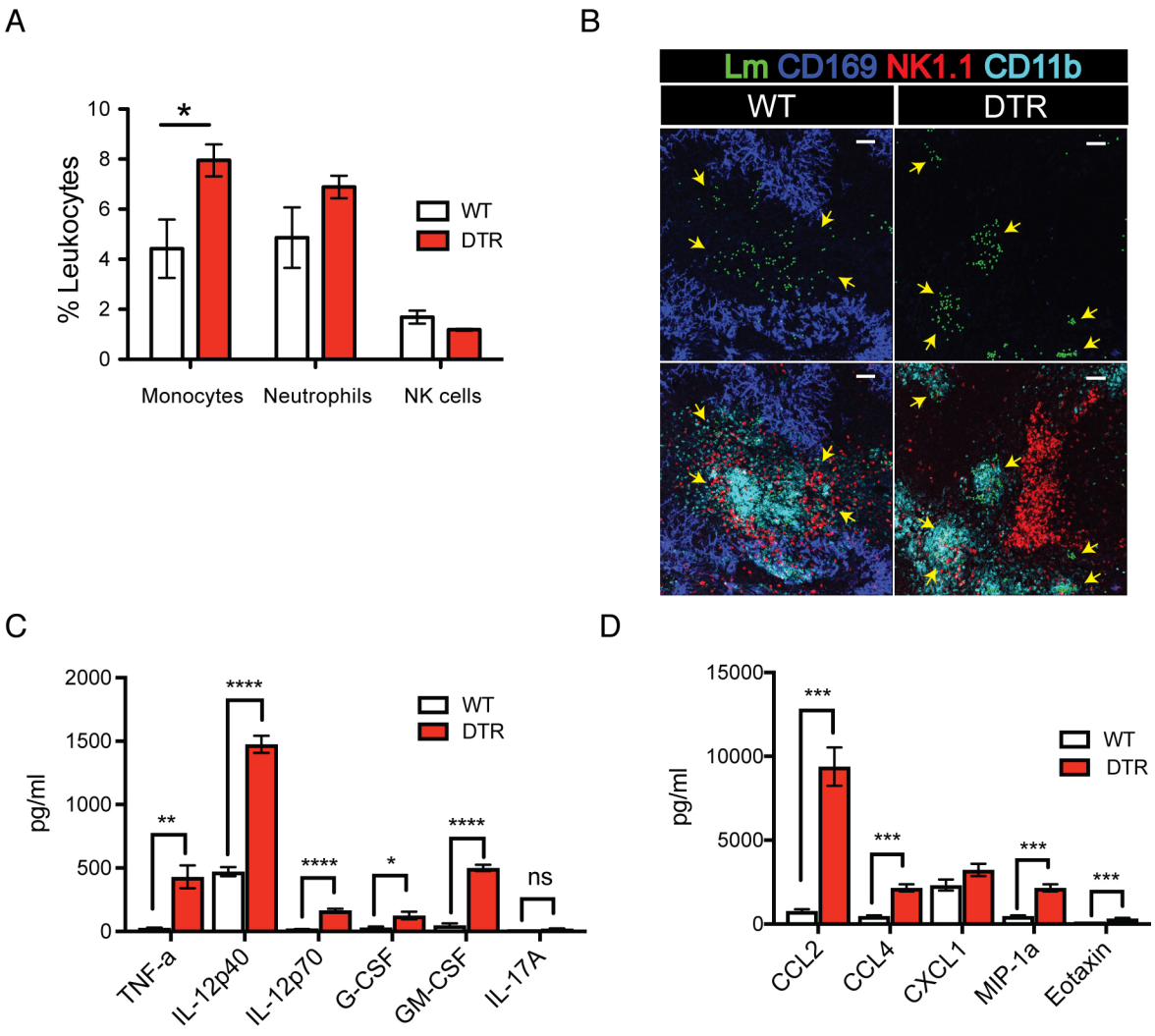
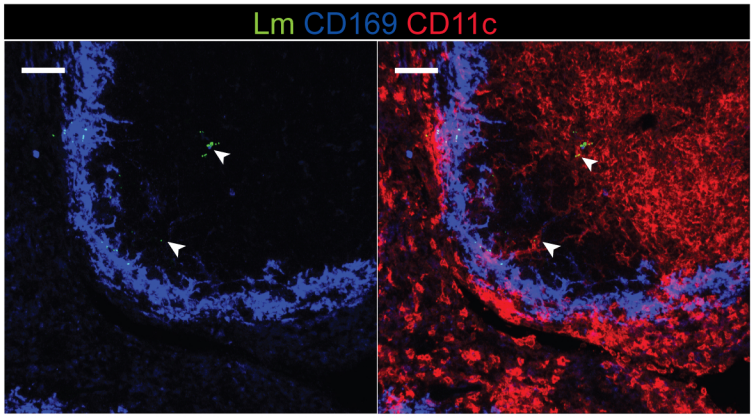


Fig. S4. CD169-DTR mice have elevated, but displaced inflammatory cytokine production. (A) Frequency of splenic monocytes (CD11b+ Ly6C+), neutrophils (CD11b+ Ly6G+), and NK cells (CD11b+ NK1.1+) in spleens of WT mice or CD169-DTR mice at 24 hpi. Shown as mean ± SEM, n=4 (*p<0.05, Student T-test). (B) Confocal microscopy of WT mice or CD169-DTR mice at 24 hpi. Spleen sections were stained for L.m. (green), CD169 (blue), NK1.1 (red) and CD11b (light blue). L.m. rendered using Spots and are indicated in green by yellow arrows. Scale bar, 50 μm. (C-D) Multiplex luminex assay of (C) inflammatory cytokines and (D) chemokines quantified from spleen lysate of WT mice or CD169-DTR mice at 24 hpi. Shown as mean ± SEM, n=4 (*p<0.05, **p<0.005, ****p<0.0001, Student T test). All data are representative of 2-4 separate experiments with 3-4 mice per group.

A



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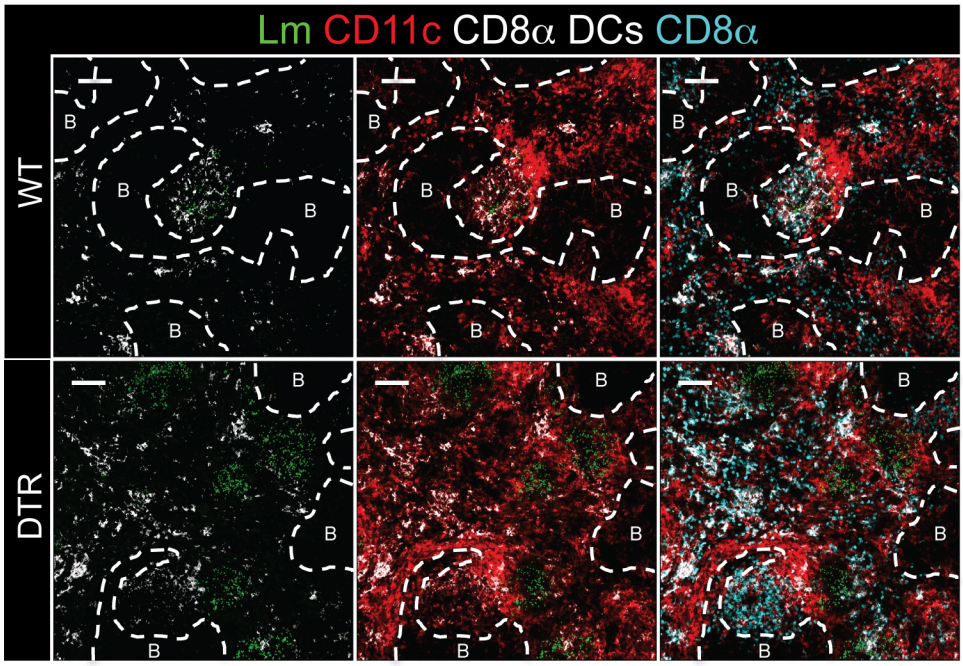


Fig. S5. CD169+ macrophages recruit CD8α+ DCs to early sites of infection. (A) Confocal images of WT mice at 12 hpi. Spleen sections stained for Lm (green), CD169 (blue) and CD11c (red). Colocalization of CD11c and CD8α is shown in white. Scale bar, 50 μm. (B) Confocal images of spleen sections from WT or CD169-DTR mice 15 hpi. Stained for L.m. (green), CD11c (red), and CD8α (light blue). Colocalization of CD8α and CD11c shown in white and L.m. rendered using Spots. Scale bar, 70-80 μm. All confocal data are representative of 3 separate experiments with 2-4 mice per group.

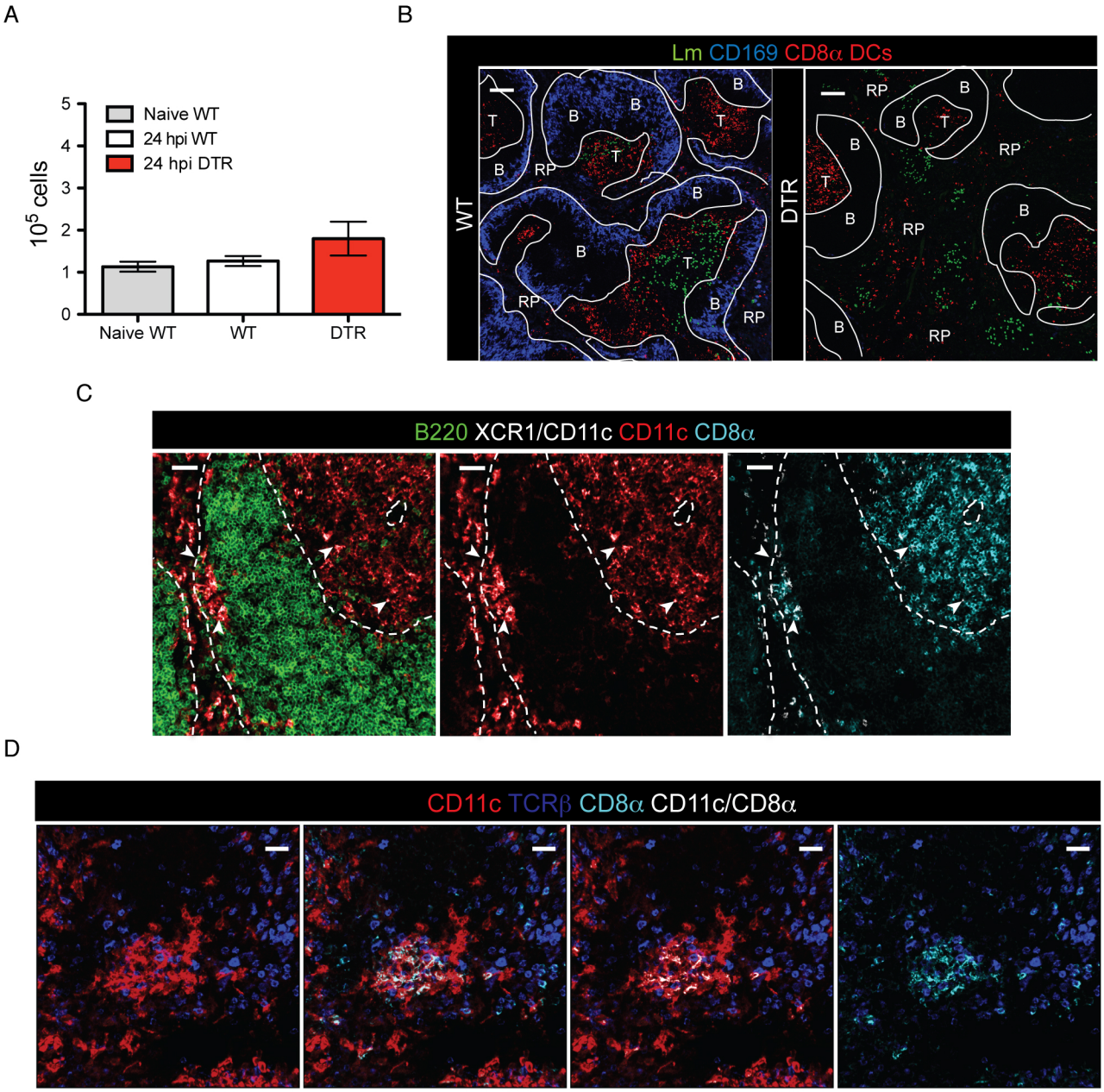


Fig. S6. Characterization of CD8α+ DCs in CD169-DTR mice. (A) FACs quantification of CD8α+ DCs in WT or CD169-DTR mice. Shown as mean ± SEM, n=3. (B) Confocal images of CD8α+ DCs in WT mice or CD169-DTR mice at 24 hpi. L.m. are shown in green, CD169 stain is shown in blue and colocalization of CD11c and CD8α is shown in red. Lm rendered using Spots. Scale bar, 100 μm. (C) Confocal image of WT spleen section 6 hpi. Stained for XCR1 and CD11c. Colocalization of XCR1 and CD11c shown in white. CD11c, CD8α, and B220 are shown in red, light blue,and green, respectively. Scale bar, 30 μm. (D) Confocal image of WT spleen section 6 hpi. Stained for CD11c (red), TCRβ (dark blue), and CD8α (light blue). Colocalization of CD11c and CD8α shown in white. Scale bar, 30 μm. All data are representative of 3 or more separate experiments with 2-3 mice per group.

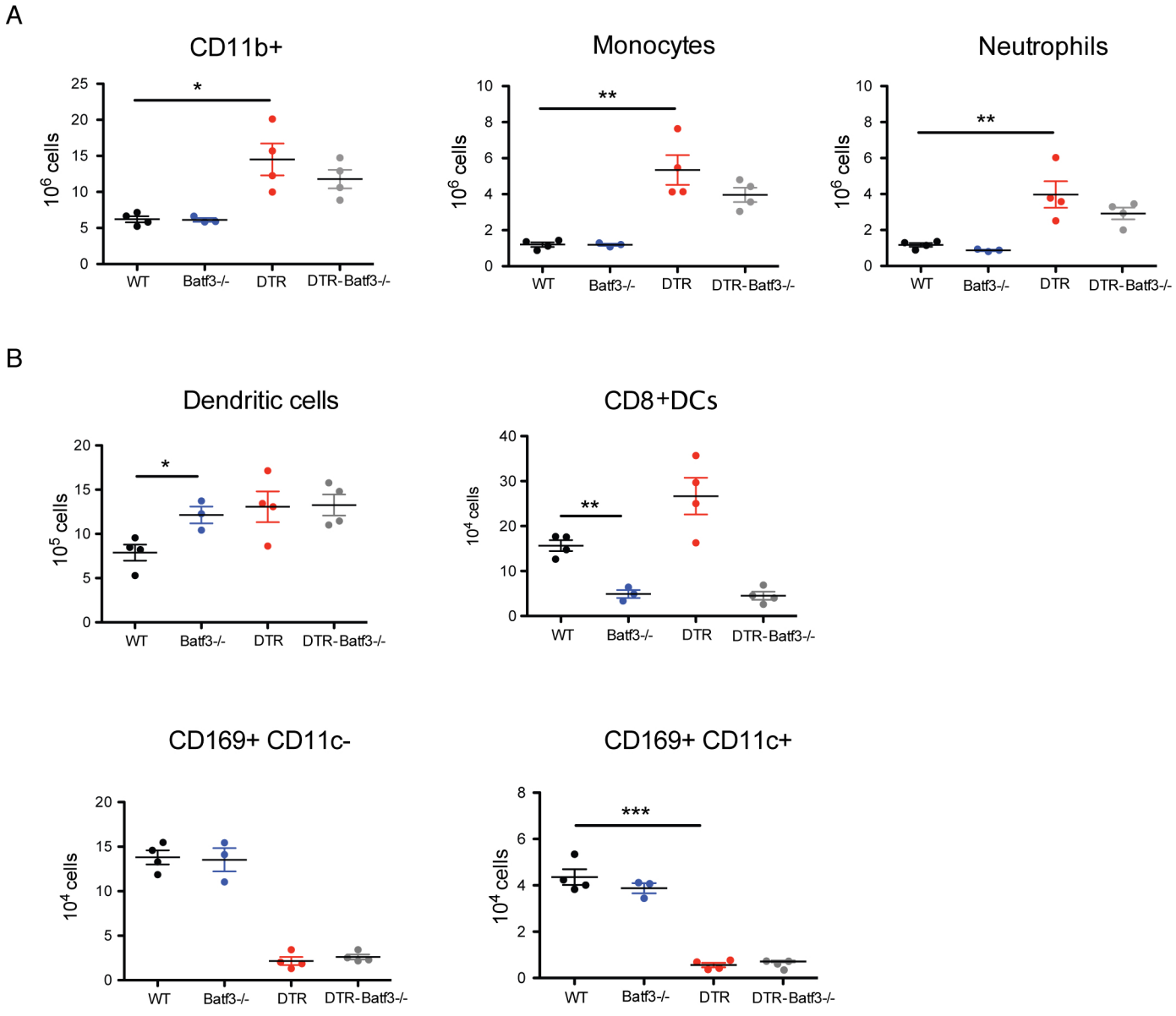
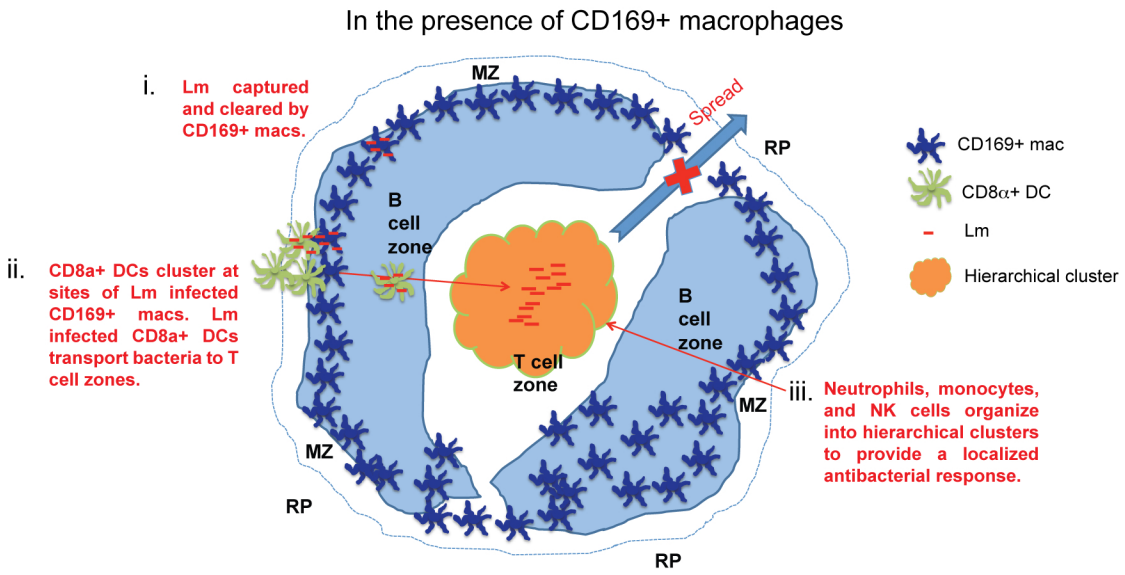


Fig. S7. Myeloid cell differentials comparing *Batf3-/-* and CD169-DTR-*Batf3-/-* mice. (A) Total number of CD11b+ cells, monocytes (CD11b+ Ly6C+), and neutrophils (CD11b+ Ly6G+) in the spleens of WT, CD169-DTR, *Batf3-/-*, or CD169-DTR-*Batf3-/-* mice at 24 hpi. Each symbol represents an individual mouse shown as mean ± SEM. (B) Total number of DCs and CD169+ macrophages in the spleens of WT, CD169-DTR, *Batf3-/-*, or CD169-DTR--mice at 24 hpi. Each symbol represents an individual mouse shown as mean ± SEM (* $P<0.05$, ** $P<0.005$, *** $P<0.0005$, One-way ANOVA). All data are representative of 3 or more separate experiments with 3-4 mice per group.

A



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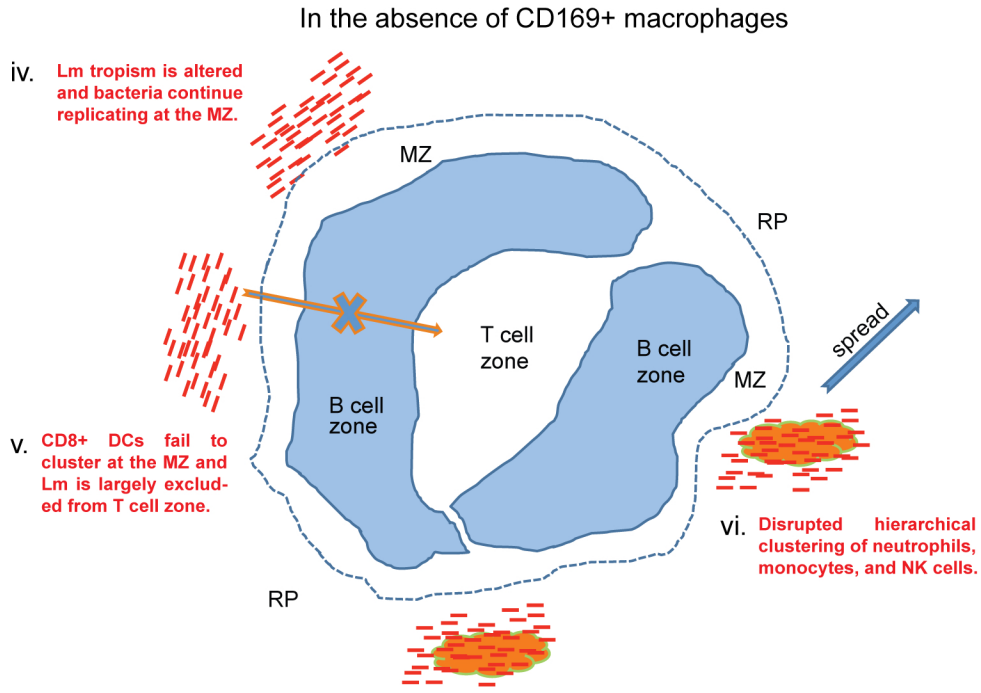

Fig. S8. Model depicting the role of CD169+ macrophages following Lm infection. (A) Top panel: (i) Following L.m. infection, bacteria are rapidly engulfed by CD169+ macrophages. The majority of bacteria are eliminated by these macrophages. However, L.m. escape from the phagolysosome initiates (ii) the recruitment of CD8α+ DCs cluster around Lm infected CD169+ macrophages, which results in the transfer of bacteria from CD169+ macrophage to CD8α+ DCs. The CD8α+ DCs then transport the bacteria to the T cell zones, where Lm continues to replicate for a brief period. Under these circumstances (iii) Lm replication is confined to splenic T cell zones where dense hierarchical clustering of neutrophils, monocytes and NK cells takes place preventing continued bacterial replication. (B) Bottom panel: In the absence of CD169+ macrophages (iv) L.m. continues to rapidly replicate unchecked in the splenic marginal zone and red pulp. (v) CD8α+ DCs fail to cluster around the L.m.-infected CD169+ macrophages. Consequently, the transfer of bacteria to the CD8 α+ DCs is abrogated, which disrupts the translocation of L.m. to the T cell zones. (vi) As a result of improper L.m. localization to the T cell zones the hierarchical clustering of innate immune cells is markedly disrupted resulting in further bacterial propagation within the spleen and spread to peripheral organs.

Table S1. Top 15 GO enriched biological terms up-regulated in CD169+ macrophages versus red pulp macrophages in steady-state

ID	Title	Number of hits	P-value	Hit names
GO:0002376	Immune system process	110	3.55E-24	Ace,Adam8,Adgrg3,Adora3,Alox5,Alox5ap,Ank,Aqp9,Bik,Btla,Camp,Ccl3,Ccl5,Ccl6,Cd24a,Cd34,Cd37,Cd40,Cd7,Cd74,Cdkn1a,Chil1,Clec10a,Clnk,Cnn2,Coro1a,Csf1,Csf2,Ctsw,Cx3cr1,Cxcl16,Cxcr2,Cyp4f18,Dtx1,Fas,Fcer1a,Ffar2,Flt3,Gata3,Gmnn,Gpr183,Gzma,Gzmb,H2DMb2,H2Eb1,H2Ob,Hdc,Hoxa7,Ifi205,Il18r1,Il18rap,Il1rl1,Il1rn,Il23r,Il2ra,Il6,Il7r,Itgam,Kit,Klra17,Klre1,L1cam,Lmo1,Lmo4,Lta,Ltb,Ltb4r1,Ltf,Mmp12,Mmp9,Ndrg1,Nxn,Osm,Pglyrp1,Pim2,Plac8,Prg2,Ptger3,Ptgr1,Ptpn7,Ptprcap,Rab27a,Rarg,Relb,Retnlg,Rorc,Runx2,S100a10,S100a8,S100a9,Samsn1,Sema4a,Sema4d,Sema7a,Serpinb1a,Sh2d3c,Sigirr,Siglecf,Socs1,Spp1,Srgn,St14,Stap1,Timeless,Tmem176a,Tmem176b,Tnfsf9,Traf1,Tspan32,Txk
GO:0001775	Cell activation	74	3.94E-22	Adgrg3,Adora3,Bik,Btla,Ccl3,Ccl5,Cd24a,Cd37,Cd40,Cd7,Cd74,Cdkn1a,Chil1,Coro1a,Csf1,Csf2,Cx3cr1,Cxcl16,Cxcr2,Dtx1,Fas,Ffar2,Flt3,Gata3,Gpr183,Gzma,Gzmb,H2Ob,Hdc,Il18r1,Il18rap,Il1rl1,Il2ra,Il7r,Itgam,Itgb3,Kit,Klre1,Lmo1,Lta,Ltb,Ltb4r1,Mmp12,Ndrg1,Osm,Pim2,Plac8,Ptger3,Ptpn7,Ptprcap,Rab27a,Rarg,Relb,Retnlg,Rorc,Runx2,S100a6,Samsn1,Sema4a,Sema4d,Sema7a,Sh2d3c,Sigirr,Siglecf,Socs1,Spp1,Srgn,St14,Timeless,Tmem176a,Tnfsf9,Traf1,Tspan32,Txk
GO:0006955	Immune response	79	3.96E-22	Ace,Adora3,Alox5,Alox5ap,Btla,Camp,Ccl3,Ccl5,Ccl6,Cd24a,Cd34,Cd37,Cd40,Cd7,Cd74,Chil1,Clec10a,Clnk,Coro1a,Csf2,Ctsw,Cx3cr1,Cxcl16,Cxcr2,Fas,Fcer1a,Gata3,Gpr183,Gzma,Gzmb,H2-DMb2,H2-Eb1,H2Ob, Hdc, Ifi205, Il18r1, Il18rap, Il1rl1,Il1rn,Il23r,Il2ra,Il6,Il7r,Itgam,Kit,Klra17,Klre1,Lmo4,Lta,Ltb,Ltb4r1,Ltf,Mmp9,Ndrg1,Nxn,Osm,Pglyrp1,Plac8,Prg2,Ptger3,Ptprcap,Rab27a,Rarg,Relb,Rorc,S100a8,S100a9,Sema4a,Sema4d,Sema7a,Serpinb1a,Sh2d3c,Sigirr,Siglecf,Socs1,Spp1,St14,Tnfsf9,Txk
GO:0045321	Leukocyte activation	70	1.84E-21	Adgrg3,Adora3,Bik,Btla,Ccl3,Cd24a,Cd37,Cd40,Cd7,Cd74,Cdkn1a,Chil1,Coro1a,Csf1,Csf2,Cx3cr1,Cxcl16,Cxcr2,Dtx1,Fas,Ffar2,Flt3,Gata3,Gpr183,Gzmb,H2Ob,Hdc,Il18r,Il18rap,Il1rl1,Il2ra,Il7r,Itgam,Kit,Klre1,Lmo1,Lta,Ltb,Ltb4r1,Mmp12,Ndrg1,Osm,Pim2,Plac8,Ptger3,Ptpn7,Ptprcap,Rab27a,Rarg,Relb,Retnlg,Rorc,Runx2,Samsn1,Sema4a,Sema4d,Sema7a,Sh2d3c,Sigirr,Siglecf,Socs1,Spp1,Srgn,St14,Timeless,Tmem176a,Tnfsf9,Traf1,Tspan32,Txk
GO:0001817	Regulation of cytokine production	54	7.21E-19	Adora3,Anxa1,Btla,Camp,Ccl3,Cd37,Cd40,Cd7,Chil1,Coro1a,Csf2,Cxcl16,Cxcr2,Fas,Ffar2,Flt3,Gata3,Gzma,Gzmb,H2Ob,Hdc,Il18r1,Il18rap,Il1rl1,Il1rn,Il2ra,Il6,Itgam,Kit,Klra17,Klre1,Ltb,Ltb4r1,Mmp9,Osm,Pglyrp1,Ptger3,Ptprcap,Ramp1,Relb,Rorc,Sema4a,Sema4d,Sema7a,Serpinb1a,Sh2d3c,Sigirr,Socs1,Spp1,Srgn,Tnfsf9,Traf1,Txk,Unc119
GO:0001816	Cytokine production	55	4.74E-18	Adora3,Anxa1,Btla,Camp,Ccl3,Cd37,Cd40,Cd7,Chil1,Coro1a,Csf2,Cxcl16,Cxcr2,Fas,Ffar2,Flt3,Gata3,Gzma,Gzmb,H2Ob,Hdc,Il18r1,Il18rap,Il1rl1,Il1rn,Il2ra,Il6,Itgam,Kit,Klra17,Klre1,Ltb,Ltb4r1,Mmp9,Osm,Pglyrp1,Ptger3,Ptprcap,Ramp1,Rarg,Relb,Rorc,Sema4a,Sema4d,Sema7a,Serpinb1a,Sh2d3c,Sigirr,Socs1,Spp1,Srgn,Tnfsf9,Traf1,Txk,Unc119
GO:0006952	Defense response	74	6.41E-18	Ace,Adam8,Adora3,Alox5,Alox5ap,Anxa1,Btla,Camp,Ccl3,Ccl5,Ccl6,Cd24a,Cd40,Cd7,Clec10a,Csf1,Csf2,Csrp2,Ctsw,Cx3cr1,Cxcl16,Cxcr2,Fas,Fcer1a,Ffar2,Gata3,Gzma,H2DMb2,H2Eb1,Hdc,Il18r1,Il18rap,Il1rl1,Il1rn,Il23r,Il2ra,Il6,Kit,Klra17,Klre1,Lta,Ltb,Ltb4r1,Ltf,Mcpt2,Mmp12,Mmp9,Nxn,Osm,Pglyrp1,Pkp3,Prdx6,Prg2,Pstpip1,Ptger3,Ptgr1,Ptprcap,Rab27a,Rarg,Relb,Rem1,Retnlg,S100a8,S100a9,Sema4d,Sema7a,Serpinb1a,Sigirr,Siglecf,Socs1,Spp1,Spry2,Srgn,Traf1
GO:0006954	Inflammatory response	57	3.05E-17	Ace,Adam8,Adora3,Alox5,Alox5ap,Anxa1,Btla,Camp,Ccl3,Ccl5,Ccl6,Cd40,Cd7,Clec10a,Csf1,Csf2,Csrp2,Cx3cr1,Cxcl16,Cxcr2,Fas,Fcer1a,Ffar2,Gata3,Gzma,H2DMb2,Hdc,Il18r1,Il1rl1,Il1rn,Il2ra,Il6,Lta,Ltb4r1,Mcpt2,Mmp12,Mmp9,Osm,Pkp3,Prdx6,Prg2,Pstpip1,Ptger3,Ptgr1,Rarg,Relb,Rem1,Retnlg,S100a8,S100a9,Sema7a,Sigirr,Siglecf,Socs1,Spp1,Srgn,Traf1
GO:0009611	Response to wounding	71	2.36E-16	Ace,Adam8,Adora3,Alox5,Alox5ap,Anxa1,Aqp3,Btla,Camp,Ccl3,Ccl5,Ccl6,Cd40,Cd7,Clec10a,Csf1,Csf2,Csrp2,Cx3cr1,Cxcl16,Cxcr2,Emp3,F5,Fas,Fcer1a,Ffar2,Gata3,Gzma,H2DMb2,Hdc,Hs3st1,Il18r1,Il1rl1,Il1rn,Il2ra,Il6,Itgam,Itgb3,Lta,Ltb4r1,Mcpt2,Mmp12,Mmp9,Ndrg1,Osm,Pkp3,Prdx6,Prg2,Pstpip1,Ptger3,Ptgr1,Rab27a,Rarg,Relb,Rem1,Retnlg,S100a4,S100a8,S100a9,Sema4d,Sema7a,Sigirr,Siglecf,Socs1,Spint1,Spp1,Srgn,Thbs1,Traf1,Tspan32,Vim
GO:0002521	Leukocyte differentiation	48	4.02E-15	Adam8,Adgrg3,Aqp9,Bik,Btla,Cd24a,Cd40,Cd74,Coro1a,Csf1,Csf2,Dtx1,Fas,Ffar2,Flt3,Gata3,Gmnn,Gpr183,Il18r1,Il1rl1,Il2ra,Il7r,Itgam,Kit,Lmo1,Lta,Ltb,Ltb4r1,Osm,Ptpn7,Rarg,Relb,Retnlg,Rorc,Runx2,S100a8,S100a9,Samsn1,Sema4a,Sema4d,Sh2d3c,Socs1,Spp1,Tmem176a,Tmem176b,Tnfsf9,Traf1,Txk
GO:0046649	Lymphocyte activation	54	6.90E-15	Adgrg3,Bik,Btla,Ccl3,Cd24a,Cd37,Cd40,Cd7,Cd74,Cdkn1a,Coro1a,Csf2,Cxcl16,Dtx1,Fas,Flt3,Gata3,Gpr183,Gzmb,Hdc,Il18r1,Il1rl1,Il2ra,Il7r,Itgam,Kit,Klre1,Lmo1,Lta,Ltb,Ltb4r1,Osm,Pim2,Ptpn7,Ptprcap,Rab27a,Rarg,Relb,Rorc,Runx2,Samsn1,Sema4a,Sema4d,Sema7a,Sh2d3c,Sigirr,Socs1,Spp1,St14,Timeless,Tnfsf9,Traf1,Tspan32,Txk
GO:0002520	Immune system development	61	1.03E-14	Adam8,Adgrg3,Ank,Aqp9,Bik,Btla,Ccl5,Cd24a,Cd34,Cd40,Cd74,Coro1a,Csf1,Csf2,Dtx1,Fas,Ffar2,Flt3,Gata3,Gmnn,Gpr183,Hdc,Hoxa7,Il18r1,Il1rl1,Il2ra,Il7r,Itgam,Kit,Klra17,L1cam,Lmo1,Lmo4,Lta,Ltb,Ltb4r1,Mmp9,Osm,Ptpn7,Rarg,Relb,Retnlg,Rorc,Runx2,S100a8,S100a9,Samsn1,Sema4a,Sema4d,Sh2d3c,Socs1,Spp1,Srgn,St14,Stap1,Tmem176a,Tmem176b,Tnfsf9,Traf1,Tspan32,Txk
GO:0080134	Regulation of response to stress	53	1.11E-14	Ace,Adam8,Adora3,Anxa1,Camp,Ccl3,Ccl5,Cd40,Cd7,Clec10a,Coro1a,Csf2,Cxcl16,Cxcr2,F5,Ffar2,Flt3,Gata3,Gzma,Hdc,Il18rap,Il1rl1,Il1rn,Il2ra,Il6,Itgb3,Kit,Klre1,Ltb,Ltb4r1,Ltf,Mmp12,Nxn,Osm,Pglyrp1,Pkp3,Prdx6,Ptger3,Rab27a,Relb,S100a6,S100a9,Sema4d,Sema7a,Serpinb1a,Sh2d3c,Sigirr,Siglecf,Socs1,Spp1,Srgn,Thbs1,Traf1
GO:0002682	Regulation of immune system process	61	1.31E-14	Ace,Adora3,Ank,Btla,Camp,Ccl3,Ccl5,Cd24a,Cd37,Cd40,Cd7,Cd74,Cdkn1a,Chil1,Cnn2,Coro1a,Csf1,Csf2,Cxcl16,Cxcr2,Fas,Flt3,Gata3,Gmnn,Gpr183,Gzmb,Hdc,Il18rap,Il1rl1,Il1rn,Il2ra,Itgam,Kit,Klre1,Lta,Ltb,Ltb4r1,Ndrg1,Nxn,Osm,Ptger3,Ptprcap,Rab27a,Relb,Retnlg,Rorc,Runx2,S100a10,S100a9,Sema4a,Sema4d,Sema7a,Serpinb1a,Sh2d3c,Sigirr,Socs1,Spp1,St14,Traf1,Tspan32,Txk
GO:0031347	Regulation of defense response	41	1.47E-14	Ace,Adam8,Adora3,Anxa1,Camp,Ccl3,Ccl5,Cd7,Clec10a,Csf2,Cxcl16,Ffar2,Gata3,Gzma,Hdc,Il18rap,Il1rl1,Il1rn,Il2ra,Il6,Klre1,Ltb,Ltb4r1,Ltf,Mmp12,Nxn,Osm,Pkp3,Prdx6,Ptger3,Rab27a,Relb,Sema4d,Sema7a,Serpinb1a,Sigirr,Siglecf,Socs1,Spp1,Srgn,Traf1

Table S2. Top 15 GO enriched biological processes up-regulated in CD169 + macrophages during infection versus steady-state

ID	Title	Number of hits	P-value	Hit names
GO:0002376	Immune system process	138	2.21E-37	Abcb1a,Adar,Adprm,Anxa2,Arg2,Batf,Bcl2l11,Bst1,Bst2,Casp1,Casp4,Ccl12,Ccnd2,Cd274,Cd40,Cd69,Cdkn1a,Chil1,Cited2,Cldn1,Clec4d,Csf2,Cx3cl1,Cxcl1,Cxcl10,Cxcl11,Cxcl3,Cxcr5,Dhx58,Dusp16,Eif2ak2,Epb41,Ets2,Etv6,Fas,Fcgr4,Fpr2,Gab2,Gadd45b,Gbp2,Gbp3,Gm12185,Gm9845,Gpr84,H2K1,H2T24,Hdc,Hipk2,Hmox1,Hrh2,Hsp90aa1,Hsph1,Htt,Ifi205,Ifi44,Ifih1,Ifit2,Ifng,Iigp1,Il12a,Il12b,Il15,Il15ra,Il1a,Il1rn,Il21r,Il27,Il2ra,Il4ra,Il6,Iqgap1,Irf7,Irgm1,Irgm2,Itga4,Kat2b,Kdr,Klf6,Lgals9,Lipg,Lrg1,Map2k1,Max,Mmp13,Ms4a6d,Msr1,Mvp,Myd88,Nampt,Ncf1,Nfkbib,Nfkbie,Nlrp3,Nod2,Notch1,Oas1b,Oasl2,Peli1,Phf11d,Phlpp1,Plscr1,Pml,Ppp1r15b,Ptpn1,Rapgef2,Rasgrp1,Rhbdf2,Ripk2,Rsad2,S100a9,Samsn1,Satb1,Serpinb9,Siglec1,Skil,Slfn3,Slfn5,Slfn8,Sos1,Sp110,Spred1,Srgn,Stat1,Stat2,Stat3,Stip1,Taf4b,Tapbp,Tifa,Timeless,Tnfsf15,Tnfsf4,Tnfsf8,Traf6,Trex1,Trim21,Usp18,Wars
GO:0051707	Response to other organism	89	9.82E-32	Adar,Arg2,Bcl2l11,Bst2,C130026I21Rik,Carhsp1,Casp1,Casp4,Ccl12,Ccnd2,Cd274,Cd40,Cd69,Cldn1,Clec4e,Csf2,Cx3cl1,Cxcl1,Cxcl10,Cxcl11,Cxcl3,Cxcr5,Dhx58,Eif2ak2,Fas,Gadd45b,Gbp3,Gm12185,Gm5431,Gm9845,Gpr84,Gvin1,H2K1,H2T24,Hdc,Hrh2,Ifi44,Ifih1,Ifit1,Ifit2,Ifit3b,Ifng,Iigp1,Il12a,Il12b,Il15,Il15ra,Il1a,Il1rn,Il27,Il2ra,Il4ra,Il6,Irf7,Irgm1,Irgm2,Lrg1,Msr1,Myd88,Ncf1,Nfkbib,Nlrp3,Nod2,Notch1,Oas1b,Oas3,Oasl1,Oasl2,Olfr56,Pfkfb3,Plscr1,Rhbdf2,Ripk2,Rsad2,S100a9,Serpinb9,Slfn5,Slfn8,Sp110,Srgn,Stat1,Stat3,Tapbp,Tifa,Tnfsf4,Trim21,Usp18,Xdh,Zbp1
GO:0009607	Response to biotic stimulus	91	4.25E-31	Adar,Arg2,Bcl2l11,Bst2,C130026I21Rik,Carhsp1,Casp1,Casp4,Ccl12,Ccnd2,Cd274,Cd40,Cd69,Cited2,Cldn1,Clec4e,Csf2,Cx3cl1,Cxcl1,Cxcl10,Cxcl11,Cxcl3,Cxcr5,Dhx58,Eif2ak2,Fas,Gadd45b,Gbp3,Gm12185,Gm5431,Gm9845,Gpr84,Gvin1,H2K1,H2T24,Hdc,Hrh2,Ifi44,Ifih1,Ifit1,Ifit2,Ifit3b,Ifng,Iigp1,Il12a,Il12b,Il15,Il15ra,Il1a,Il1rn,Il27,Il2ra,Il4ra,Il6,Irf7,Irgm1,Irgm2,Lrg1,Msr1,Myd88,Ncf1,Nfkbib,Nlrp3,Nod2,Notch1,Oas1b,Oas3,Oasl1,Oasl2,Olfr56,Pfkfb3,Plscr1,Rhbdf2,Ripk2,Rsad2,S100a9,Serpinb9,Slfn5,Slfn8,Sp110,Srgn,Stat1,Stat3,Tapbp,Tifa,Tnfsf4,Trim21,Ubxn4,Usp18,Xdh,Zbp1
GO:0006952	Defense response	94	1.70E-27	Abcb1a,Adar,Arg2,Bcl2l11,Bst2,Casp1,Casp4,Ccl12,Cd274,Cd40,Cd69,Cldn1,Clec4e,Csf2,Cx3cl1,Cxcl1,Cxcl10,Cxcl11,Cxcl3,Dhx58,Eif2ak2,Fas,Fpr2,Gab2,Gbp2,Gbp3,Gem,Gm12185,Gm5431,Gm9845,Gm9847,Gpr84,H2K1,H2T24,Hdc,Hmox1,Hrh2,Ifi44,Ifih1,Ifit2,Ifng,Iigp1,Il12a,Il12b,Il15,Il15ra,Il1a,Il1rn,Il27,Il2ra,Il4ra,Il6,Irf7,Irgm1,Irgm2,Itga4,Itga5,Lipg,Lrp8,Mapk13,Msr1,Myd88,Ncf1,Nfkbib,Nlrp3,Nod2,Oas1b,Peli1,Pfkfb3,Phlpp1,Plscr1,Pml,Procr,Rasgrp1,Rhbdf2,Ripk2,Rsad2,S100a9,Serpinb9,Siglec1,Slc7a11,Sp110,Srgn,Stat1,Stat3,Tapbp,Tifa,Tnfsf4,Tnip3,Tpst1,Traf6,Trim21,Usp18,Zbp1
GO:0006955	Immune response	90	3.17E-26	Adar,Adprm,Arg2,Batf,Bcl2l11,Bst2,Casp1,Casp4,Cd274,Cd40,Cd69,Chil1,Clec4d,Csf2,Cx3cl1,Cxcl1,Cxcl10,Cxcl11,Cxcr5,Dhx58,Eif2ak2,Epb41,Fas,Fcgr4,Gab2,Gadd45b,Gbp2,Gbp3,Gm12185,Gm9845,Gpr84,H2-K1,H2-T24,Hdc,Hmox1,Hrh2,Hsph1,Ifi205,Ifi44,Ifih1,Ifit2,Ifng,Iigp1,Il12a,Il12b,Il15,Il15ra,Il1a,Il1rn,Il21r,Il2ra,Il4ra,Il6,Irf7,Irgm1,Irgm2,Itga4,Map2k1,Msr1,Myd88,Nfkbie,Nlrp3,Nod2,Notch1,Oasl2,Peli1,Pml,Rasgrp1,Rhbdf2,Ripk2,Rsad2,S100a9,Serpinb9,Siglec1,Slfn3,Slfn5,Sp110,Stat1,Stat2,Stat3,Stip1,Tapbp,Tifa,Tnfsf15,Tnfsf4,Traf6,Trex1,Trim21,Usp18,Wars
GO:0051704	Multi-organism process	96	3.20E-26	Adar,Areg,Arg2,Bcl2l11,Bst2,C130026I21Rik,Carhsp1,Casp1,Casp4,Ccl12,Ccnd2,Cd274,Cd40,Cd69,Cldn1,Clec4e,Csf2,Cx3cl1,Cxcl1,Cxcl10,Cxcl11,Cxcl3,Cxcr5,Dhx58,Eif2ak2,Fas,Gadd45b,Gbp3,Gm12185,Gm5431,Gm9845,Gpr84,Gpr85,Gvin1,H2-K1,H2-T24,Hdc,Hrh2,Hsp90ab1,Ifi44,Ifih1,Ifit1,Ifit2,Ifit3b,Ifng,Iigp1,Il12a,Il12b,Il15,Il15ra,Il1a,Il1rn,Il27,Il2ra,Il4ra,Il6,Irf7,Irgm1,Irgm2,Itga4,Klf6,Lrg1,Msr1,Myd88,Ncf1,Nfkbib,Nlrp3,Nod2,Notch1,Oas1b,Oas3,Oasl1,Oasl2,Olfr56,Pfkfb3,Plscr1,Pml,Rhbdf2,Ripk2,Rsad2,S100a9,Serpinb9,Slfn5,Slfn8,Sp110,Srgn,Stat1,Stat3,Tapbp,Tifa,Tnfsf4,Trim21,Trim25,Usp18,Xdh,Zbp1
GO:0009617	Response to bacterium	61	3.76E-24	Adar,Arg2,Bcl2l11,Carhsp1,Casp1,Casp4,Ccl12,Ccnd2,Cd274,Cd40,Clec4e,Csf2,Cx3cl1,Cxcl1,Cxcl10,Cxcl11,Cxcl3,Cxcr5,Fas,Gbp3,Gm9845,Gpr84,Gvin1,Hdc,Hrh2,Ifit1,Ifit2,Ifng,Iigp1,Il12a,Il12b,Il15,Il1a,Il1rn,Il4ra,Il6,Irgm1,Irgm2,Lrg1,Msr1,Myd88,Ncf1,Nfkbib,Nod2,Pfkfb3,Plscr1,Rhbdf2,Ripk2,Rsad2,S100a9,Serpinb9,Slfn5,Sp110,Srgn,Stat1,Stat3,Tifa,Tnfsf4,Usp18,Xdh,Zbp1
GO:0006954	Inflammatory response	67	1.07E-21	Abcb1a,Arg2,Casp1,Casp4,Ccl12,Cd274,Cd40,Cd69,Clec4e,Csf2,Cx3cl1,Cxcl1,Cxcl10,Cxcl11,Cxcl3,Eif2ak2,Fas,Fpr2,Gab2,Gm12185,Gm9847,Gpr84,Hdc,Hmox1,Hrh2,Ifng,Iigp1,Il12a,Il12b,Il15,Il15ra,Il1a,Il1rn,Il27,Il2ra,Il4ra,Il6,Irf7,Irgm2,Itga4,Itga5,Lipg,Mapk13,Msr1,Myd88,Ncf1,Nfkbib,Nlrp3,Nod2,Peli1,Pfkfb3,Procr,Rasgrp1,Ripk2,S100a9,Serpinb9,Siglec1,Slc7a11,Srgn,Stat1,Stat3,Tnfsf4,Tnip3,Tpst1,Traf6,Trim21,Usp18
GO:0009615	Response to virus	40	1.23E-21	Adar,Bcl2l11,Bst2,C130026I21Rik,Ccl12,Cd40,Cd69,Cldn1,Cx3cl1,Cxcl1,Cxcl10,Dhx58,Eif2ak2,Fas,Gadd45b,Gm12185,Ifih1,Ifit2,Ifit3b,Ifng,Il12b,Il15,Il2ra,Irf7,Msr1,Myd88,Nlrp3,Notch1,Oas1b,Oas3,Oasl1,Oasl2,Plscr1,Rsad2,Stat1,Stat3,Tapbp,Tnfsf4,Trim21,Usp18
GO:0009611	Response to wounding	83	1.13E-20	Abcb1a,Anxa2,Areg,Arg2,Casp1,Casp4,Ccl12,Cd274,Cd40,Cd69,Cited2,Clec4e,Csf2,Cx3cl1,Cxcl1,Cxcl10,Cxcl11,Cxcl3,Eif2ak2,Epb41,Fas,Fn1,Fpr2,Gab2,Gja4,Gjb2,Gm12185,Gm9845,Gm9847,Gpr84,Hdc,Hmox1,Hrh2,Ifng,Iigp1,Il12a,Il12b,Il15,Il15ra,Il1a,Il1rn,Il27,Il2ra,Il4ra,Il6,Irf7,Irgm2,Itga4,Itga5,Kdr,Klf6,Lipg,Lrp8,Map2k1,Mapk13,Mmp13,Msr1,Myd88,Ncf1,Nfkbib,Nlrp3,Nod2,Notch1,Peli1,Pfkfb3,Procr,Rasgrp1,Ripk2,S100a9,Serpinb9,Siglec1,Slc7a11,Srgn,Stat1,Stat3,Thbs1,Tnfsf15,Tnfsf4,Tnip3,Tpst1,Traf6,Trim21,Usp18
GO:0045087	Innate immune response	42	2.19E-20	Adar,Arg2,Bcl2l11,Bst2,Casp1,Casp4,Cd274,Csf2,Cx3cl1,Cxcl1,Cxcl11,Dhx58,Eif2ak2,Gbp2,Gbp3,Hmox1,Hrh2,Ifih1,Ifit2,Iigp1,Il12a,Il12b,Il15,Il15ra,Il2ra,Irf7,Irgm1,Irgm2,Myd88,Nod2,Peli1,Pml,Rhbdf2,Ripk2,Rsad2,Serpinb9,Sp110,Stat1,Stat3,Tifa,Trim21,Usp18
GO:0080134	Regulation of response to stress	65	2.76E-20	Arg2,Bcl2l11,Casp1,Cd274,Cd40,Cited2,Csf2,Cx3cl1,Cxcl1,Cxcl3,Dhx58,Dusp16,Eif2ak2,Epb41,Etv6,Fpr2,Gab2,Gadd45b,Gm9845,Gm9847,Hdc,Hmox1,Hrh2,Hsph1,Ifih1,Il12a,Il12b,Il15,Il15ra,Il1rn,Il27,Il2ra,Il4ra,Il6,Irf7,Irgm2,Itga4,Kdm2b,Lrp8,Msr1,Myd88,Ncf1,Nlrp3,Nod2,Peli1,Pfkfb3,Phlpp1,Plscr1,Pml,Procr,Ptpn1,Rasgrp1,Ripk2,S100a9,Serpinb9,Siglec1,Sp110,Srgn,Stat1,Stat3,Thbs1,Tnfsf4,Traf6,Trim21,Usp18
GO:0001816	Cytokine production	61	4.71E-20	Arg2,Bcl2l11,Bst2,Carhsp1,Casp1,Casp4,Cd274,Cd40,Cd69,Chil1,Csf2,Cx3cl1,Cxcl10,Dusp16,Eif2ak2,Epb41,Fas,Fcgr4,Gab2,Gm9847,Gpr84,Hdc,Hmox1,Hrh2,Hsph1,Htt,Ifih1,Ifng,Il12a,Il12b,Il15,Il15ra,Il1rn,Il27,Il2ra,Il4ra,Il6,Irf7,Itga4,Mmp13,Ms4a4c,Msr1,Myd88,Nampt,Nfkbib,Nfkbie,Nlrp3,Nod2,Pfkfb3,Rasgrp1,Rhbdf2,Ripk2,Rsad2,Srgn,Stat1,Stat3,Tnfsf15,Tnfsf4,Traf6,Trim21,Usp18
GO:0006950	Response to stress	135	5.54E-20	Abcb1a,Adar,Anxa2,Areg,Arg2,Bcl2l11,Bst2,Casp1,Casp4,Ccl12,Ccnd2,Cd274,Cd40,Cd69,Cdkn1a,Cited2,Cldn1,Clec4e,Csf2,Cx3cl1,Cxcl1,Cxcl10,Cxcl11,Cxcl3,Dhx58,Dram1,Dusp16,Eif2ak2,Epb41,Etv6,Fas,Fn1,Fpr2,Gab2,Gadd45b,Gbp2,Gbp3,Gem,Gja4,Gjb2,Gm12185,Gm5431,Gm9845,Gm9847,Gpr84,H2-K1,H2-T24,Hdc,Hipk2,Hmox1,Hrh2,Hsp90aa1,Hsp90ab1,Hspa2,Hsph1,Htt,Ifi44,Ifih1,Ifit2,Ifng,Iigp1,Il12a,Il12b,Il15,Il15ra,Il1a,Il1rn,Il27,Il2ra,Il4ra,Il6,Irf7,Irgm1,Irgm2,Itga4,Itga5,Kdm2b,Kdr,Klf6,Lipg,Lrp8,Map2k1,Mapk13,Mdm2,Mfsd2a,Mmp13,Msr1,Myd88,Myo6,Nampt,Ncf1,Nfkbib,Nlrp3,Nod2,Notch1,Oas1b,Papd7,Parp3,Pde5a,Peli1,Pfkfb3,Phlpp1,Plscr1,Pml,Ppp1r15b,Procr,Ptpn1,Rab20,Rasgrp1,Rhbdf2,Ripk2,Rsad2,S100a9,Serpinb9,Siglec1,Slc7a11,Sp110,Srgn,Stat1,Stat3,Stip1,Tapbp,Thbs1,Tifa,Timeless,Tnfsf15,Tnfsf4,Tnip3,Tpst1,Traf6,Trex1,Trim21,Ubxn4,Usp18,Zbp1
GO:0031347	Regulation of defense response	47	3.19E-17	Arg2,Bcl2l11,Cd274,Csf2,Cx3cl1,Cxcl1,Cxcl3,Dhx58,Eif2ak2,Fpr2,Gm9847,Hdc,Hmox1,Hrh2,Ifih1,Il12a,Il12b,Il15,Il15ra,Il1rn,Il27,Il2ra,Il4ra,Il6,Irf7,Irgm2,Itga4,Msr1,Myd88,Ncf1,Nlrp3,Nod2,Peli1,Pfkfb3,Phlpp1,Plscr1,Pml,Ripk2,Serpinb9,Siglec1,Sp110,Srgn,Stat1,Stat3,Tnfsf4,Trim21,Usp18

Table S3. Top 15 GO enriched biological processes down-regulated in CD169 + macrophages during infection versus steady-state

ID	Title	Number of hits	P-value	Hit names
GO:0044237	Cellular metabolic process	152	3.17E-07	Abcd2,Acaa2,Acp6,Acyp1,Ak8,Akr7a5,Aldoc,Anapc11,Anapc13,Aqp3,Arl4d,Asb2,Ascc1,Asnsd1,Atp5g2,Atp5h,Atraid,B230118H07Rik,B4gat1,BC026585,Bckdha,Bckdk,Cbr3,Cbx3,Cbx8,Cd1d1,Cdc26,Celf4,Cenpv,Chst12,Cox6b2,Cox7a2l,D10Jhu81e,Dbi,Dcxr,Dffb,Dguok,Dna2,Dnase1l1,Dpep2,Dusp6,Dusp7,Dut,E2f1,Ech1,Eif3f,Elk3,Entpd6,Epor,Erp29,Exosc5,Fam105a,Fbxl6,Flt3,Ftsj2,Gamt,Gba2,Gm10175,Gpi1,Gpn3,Guk1,Gusb,H2DMb2,H2afv,Hadh,Haghl,Hes6,Hist3h2ba,Hmgcl,Hoxa7,Hpgd,Hscb,Igflr1,Ilvbl,Impa2,Khk,Klhdc2,Klhl6,Klrd1,Maf1,Map2k6,Mcee,Mdp1,Med30,Mfng,Mpp6,Mri1,Mrpl34,Mrps16,Msh2,Mvk,Mxd4,Naca,Naga,Ndufa2,Ndufa6,Ndufb8,Ndufc2,Neurl2,Nmb,Nr1d1,Nsa2,Nudt14,Nudt16,Obfc1,Ormdl1,P3h2,Paip2,Paqr7,Patz1,Pdia5,Phpt1,Pigx,Pik3ip1,Pnkd,Pold4,Polr1e,Polr2e,Prr5,Ptger3,Ptgis,Ptp4a3,Ptpn18,Ptprcap,Pts,Pygo2,Ramp1,Rbfa,Rpa3,Rpl10ps3,Rpl36al,Rps13ps2,Rps16ps2,Rxrb,S100a10,Scp2,Sept4,Sgsh,Sigirr,Slc2a8,Slc35c2,Snrpg,Spr,Spsb2,Syce2,Tspyl4,Ube2e2,Uqcrh,Use1,Zfp467,Zfp61,Zfp740
GO:0008152	Metabolic process	163	2.81E-06	Abcd2,Acaa2,Acp6,Acyp1,Ak8,Akr7a5,Aldoc,Anapc11,Anapc13,Aqp3,Arl4d,Asb2,Ascc1,Asnsd1,Atp5g2,Atp5h,Atraid,B230118H07Rik,B4gat1,BC026585,Bckdha,Bckdk,Car9,Cbr3,Cbx3,Cbx8,Cd1d1,Cdc26,Celf4,Cenpv,Chst12,Cnpy2,Cox6b2,Cox7a2l,Cuta,D10Jhu81e,Dbi,Dcxr,Dffb,Dguok,Dna2,Dnase1l1,Dpep2,Dpm3,Dtx1,Dusp6,Dusp7,Dut,E2f1,Ebpl,Ech1,Eif3f,Elk3,Engase,Entpd6,Epor,Erp29,Exosc5,Fam105a,Fbxl6,Flt3,Ftsj2,Gamt,Gba2,Gm10175,Gpi1,Gpn3,Guk1,Gusb,H2-DMb2,H2Oa,H2afv,Hadh,Haghl,Hes6,Hist3h2ba,Hmgcl,Hoxa7,Hpgd,Hscb,Igflr1,Ilvbl,Impa2,Khk,Klhdc2,Klhl6,Klrd1,Lage3,Maf1,Map2k6,Mcee,Mdp1,Med30,Mfng,Mmp12,Mpp6,Mri1,Mrpl34,Mrps16,Msh2,Mvk,Mxd4,Naca,Naga,Ndufa2,Ndufa6,Ndufb8,Ndufc2,Neurl2,Nmb,Nr1d1,Nsa2,Nudt14,Nudt16,Obfc1,Ormdl1,P3h2,Paip2,Paqr7,Patz1,Pdia5,Phpt1,Pigx,Pik3ip1,Pnkd,Pold4,Polr1e,Polr2e,Prr5,Prss30,Ptger3,Ptgis,Ptp4a3,Ptpn18,Ptprcap,Pts,Pygo2,Ramp1,Rbfa,Rpa3,Rpl10ps3,Rpl36al,Rps13ps2,Rps16ps2,Rxrb,S100a10,Scp2,Sept4,Sgsh,Sigirr,Slc2a8,Slc35c2,Snrpg,Spr,Spsb2,Syce2,Tspyl4,Ube2e2,Uqcrh,Use1,Zfp467,Zfp61,Zfp740
GO:0022904	Respiratory electron transport chain	6	2.74E-05	Cox6b2,Cox7a2l,Ndufa6,Ndufb8,Ndufc2,Uqcrh
GO:0044282	Small molecule catabolic process	12	5.98E-05	[Abcd2,Aldoc,Bckdha,Dcxr,Ech1,Gamt,Gba2,Gpi1,Hadh,Hmgcl,Rxrb,Scp2]
GO:0022900	Electron transport chain	6	8.90E-05	[Cox6b2,Cox7a2l,Ndufa6,Ndufb8,Ndufc2,Uqcrh]
GO:0072329	Monocarboxylic acid catabolic process	7	1.12E-04	[Abcd2,Ech1,Gamt,Gba2,Hadh,Rxrb,Scp2]
GO:0042773	ATP synthesis coupled electron transport	5	1.25E-04	[Cox6b2,Ndufa6,Ndufb8,Ndufc2,Uqcrh]
GO:0042775	Mitochondrial ATP synthesis coupled electron transport	5	1.25E-04	[Cox6b2,Ndufa6,Ndufb8,Ndufc2,Uqcrh]
GO:0044281	Small molecule metabolic process	45	1.32E-04	[Abcd2,Acaa2,Ak8,Akr7a5,Aldoc,Asnsd1,Atp5g2,Atp5h,BC026585,Bckdha,Bckdk,Cenpv,Dbi,Dcxr,Dguok,Dpep2,Dusp6,Ebpl,Ech1,Entpd6,Gamt,Gba2,Gm10175,Gpi1,Guk1,Hadh,Hmgcl,Hpgd,Ilvbl,Khk,Mpp6,Mvk,Nudt14,Nudt16,Paqr7,Ptger3,Ptgis,Pts,Ramp1,Rxrb,S100a10,Scp2,Sept4,Slc2a8,Spr]
GO:0009081	Branched chain family amino acid metabolic process	4	2.01E-04	[Bckdha,Bckdk,Hmgcl,Ilvbl]
GO:0016054	Organic acid catabolic process	9	2.18E-04	[Abcd2,Bckdha,Ech1,Gamt,Gba2,Hadh,Hmgcl,Rxrb,Scp2]
GO:0046395	Carboxylic acid catabolic process	9	2.18E-04	[Abcd2,Bckdha,Ech1,Gamt,Gba2,Hadh,Hmgcl,Rxrb,Scp2]
GO:0043617	Cellular response to sucrose starvation	2	2.26E-04	[Ascc1,Nsa2]
GO:0006119	Oxidative phosphorylation	6	2.30E-04	[Cox6b2,Ndufa2,Ndufa6,Ndufb8,Ndufc2,Uqcrh]
GO:0006635	Fatty acid beta-oxidation	6	2.30E-04	[Abcd2,Ech1,Gamt,Hadh,Rxrb,Scp2]

Table S4. List of identified transcription factor candidates for up-regulated genes in CD169+ macrophages isolated from infected mice versus uninfected mice

ID	Mean CD169 12h Inf (FPKM)	Gene description	Gene symbol
ENSMUSG00000001911	3.48353	nuclear factor I/X	Nfix
ENSMUSG00000002111	211.16185	spleen focus forming virus (SFFV) proviral integration oncogene	Spi1
ENSMUSG00000002147	58.33423	signal transducer and activator of transcription 6	Stat6
ENSMUSG00000002325	58.71892	interferon regulatory factor 9	Irf9
ENSMUSG00000003154	2.33393	forkhead box J2	Foxj2
ENSMUSG00000003184	35.22463	interferon regulatory factor 3	Irf3
ENSMUSG00000003545	46.16813	FBJ osteosarcoma oncogene B	Fosb
ENSMUSG00000004040	125.16703	signal transducer and activator of transcription 3	Stat3
ENSMUSG00000004043	16.61893	signal transducer and activator of transcription 5A	Stat5a
ENSMUSG00000004661	4.19606	AT rich interactive domain 3B (BRIGHT-like)	Arid3b
ENSMUSG00000005893	1.27888	group C,member 2,nuclear receptor subfamily 2	Nr2c2
ENSMUSG00000008496	13.81689	POU domain,class 2,transcription factor 2	Pou2f2
ENSMUSG00000009739	2.94284	POU domain,class 6,transcription factor 1	Pou6f1
ENSMUSG00000019564	2.78987	AT rich interactive domain 3A (BRIGHT-like)	Arid3a
ENSMUSG00000019947	2.87096	AT rich interactive domain 5B (MRF1-like)	Arid5b
ENSMUSG00000020160	1.99312	Meis homeobox 1	Meis1
ENSMUSG00000020919	20.76732	signal transducer and activator of transcription 5B	Stat5b
ENSMUSG00000021250	239.80267	FBJ osteosarcoma oncogene	Fos
ENSMUSG00000021356	9.63148	interferon regulatory factor 4	Irf4
ENSMUSG00000022508	15.18419	B cell leukemia/lymphoma 6	Bcl6
ENSMUSG00000022556	8.74937	heat shock factor 1	Hsf1
ENSMUSG00000022641	14.45411	bobby sox homolog (Drosophila)	Bbx
ENSMUSG00000024927	52.10485	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	Rela
ENSMUSG00000025225	142.85440	nuclear factor of kappa light polypeptide gene enhancer in B cells 2,p49/p100	Nfkb2
ENSMUSG00000025498	1318.16779	interferon regulatory factor 7	Irf7
ENSMUSG00000025612	22.38609	BTB and CNC homology 1,basic leucine zipper transcription factor 1	Bach1
ENSMUSG00000025880	5.03151	SMAD family member 7	Smad7
ENSMUSG00000026565	1.11197	POU domain,class 2,transcription factor 1	Pou2f1
ENSMUSG00000026815	2.83134	growth factor independent 1B	Gfi1b
ENSMUSG00000028163	141.82010	nuclear factor of kappa light polypeptide gene enhancer in B cells 1,p105	Nfkb1
ENSMUSG00000028717	2.09910	T cell acute lymphocytic leukemia 1	Tal1
ENSMUSG00000029135	29.24672	fos-like antigen 2	Fosl2
ENSMUSG00000029771	169.35239	interferon regulatory factor 5	Irf5
ENSMUSG00000030557	11.09849	myocyte enhancer factor 2A	Mef2a
ENSMUSG00000031627	61.00421	interferon regulatory factor 2	Irf2
ENSMUSG00000031885	40.41235	core binding factor beta	Cbfb
ENSMUSG00000032053	16.06835	POU domain,associating factor 1,class 2	Pou2af1
ENSMUSG00000032998	4.96541	forkhead box J3	Foxj3
ENSMUSG00000034271	17.47371	Jun dimerization protein 2	Jdp2
ENSMUSG00000034957	3.57919	CCAAT/enhancer binding protein (C/EBP),alpha	Cebpa
ENSMUSG00000037447	125.09685	AT rich interactive domain 5A (MRF1-like)	Arid5a
ENSMUSG00000037992	14.32806	alpha,retinoic acid receptor	Rara
ENSMUSG00000039153	4.36355	runt related transcription factor 2	Runx2
ENSMUSG00000042903	3.08227	forkhead box O4	Foxo4
ENSMUSG00000047141	2.25304	zinc finger protein 654	Zfp654
ENSMUSG00000048756	3.87127	forkhead box O3	Foxo3
ENSMUSG00000052534	7.54392	pre B cell leukemia homeobox 1	Pbx1
ENSMUSG00000052684	129.88570	jun proto-oncogene	Jun
ENSMUSG00000052837	521.61200	jun B proto-oncogene	Junb
ENSMUSG00000055053	2.33381	nuclear factor I/C	Nfic
ENSMUSG00000056216	7.90125	CCAAT/enhancer binding protein (C/EBP),gamma	Cebpg
ENSMUSG00000056493	4.10002	forkhead box K1	Foxk1
ENSMUSG00000056501	99.98105	CCAAT/enhancer binding protein (C/EBP),beta	Cebpb
ENSMUSG00000057469	3.75573	E2F transcription factor 6	E2f6
ENSMUSG00000059475	3.40205	zinc finger protein 426	Zfp426
ENSMUSG00000062175	2.92555	TGFB-induced factor homeobox 2	Tgif2
ENSMUSG00000071076	74.01905	jun D proto-oncogene	Jund
ENSMUSG00000074220	1.51117	zinc finger protein 382	Zfp382

Table S5. Enriched immune-related GO terms for corresponding transcription factors in CD169+ macrophages during infection versus steady-state

ID	Title	Number of hits	P-value	Hit names
GO:0002682	**Regulation of immune system process**	28	2.30E-18	Bcl6,Cebpb,Cebpg,Fos,Foxo3,Hsf1,Irf2,Irf3,Irf4,Irf5,Irf7,Irf9,Jdp2,Junb,Meis1,Nfic,Nfkb1,Nfkb2,Pbx1,Pou2af1,Rara,Runx2,Smad7,Spi1,Stat3,Stat5a,Stat6,Tal1
GO:0002520	**Immune system development**	27	2.82E-17	Arid3a,Bcl6,Cbfb,Cebpb,Fos,Foxo3,Gfi1b,Irf2,Irf4,Irf5,Irf9,Jdp2,Junb,Meis1,Nfkb1,Nfkb2,Pbx1,Pou2af1,Pou2f1,Rara,Runx2,Spi1,Stat3,Stat5a,Stat6,Tal1,Zfp426
GO:0002376	**Immune system process**	34	2.38E-16	Arid3a,Bcl6,Cbfb,Cebpb,Cebpg,Fos,Foxo3,Gfi1b,Hsf1,Irf2,Irf3,Irf4,Irf5,Irf, Irf9,Jdp2,Junb,Meis1,Nfic,Nfkb1,Nfkb2,Pbx1,Pou2af1,Pou2f1,Rara,Rela,Runx2,Smad7,Spi1,Stat3,Stat5a,Stat6,Tal1,Zfp426
GO:0006955	**Immune response**	22	9.75E-11	Arid3a,Bcl6,Cbfb,Cebpb,Cebpg,Fos,Irf2,Irf3,Irf4,Irf5,Irf7,Irf9,Junb,Nfkb1,Nfkb2,Pou2af1,Rela,Smad7,Spi1,Stat3,Stat5a,Stat6
GO:0002697	**Regulation of immune effector process**	13	8.93E-10	Bcl6,Cebpb,Cebpg,Fos,Hsf1,Irf2,Irf3,Irf4,Irf9,Junb,Nfkb1,Stat3,Stat6
GO:0002252	**Immune effector process**	15	1.47E-09	Bcl6,Cebpb,Cebpg,Fos,Hsf1,Irf2,Irf3,Irf4,Irf9,Junb,Nfkb1,Pou2af1,Stat3,Stat5a,Stat6
GO:0050776	**Regulation of immune response**	15	2.27E-09	Bcl6,Cebpb,Cebpg,Fos,Irf2,Irf3,Irf4,Irf7,Irf9,Junb,Nfkb1,Pou2af1,Spi1,Stat3,Stat6
GO:0002440	**Production of molecular mediator of immune response**	10	1.01E-08	Bcl6,Hsf1,Irf3,Irf4,Irf9,Junb,Nfkb1,Pou2af1,Stat3,Stat6
GO:0002377	**Immunoglobulin production**	9	1.46E-08	Bcl6,Hsf1,Irf3,Irf4,Irf9,Junb,Nfkb1,Pou2af1,Stat6
GO:0002366	**Leukocyte activation involved in immune response**	9	2.60E-07	Bcl6,Fos,Irf2,Irf4,Irf9,Junb,Nfkb1,Stat5a,Stat6
GO:0002443	**Leukocyte mediated immunity**	9	2.87E-06	Bcl6,Cebpg,Fos,Irf4,Irf9,Junb,Nfkb1,Pou2af1,Stat6
GO:0045087	**Innate immune response**	9	3.61E-06	Cebpb,Cebpg,Irf2,Irf3,Irf4,Irf7,Irf9,Nfkb1,Stat3
GO:0002285	**Lymphocyte activation involved in immune response**	7	4.14E-06	Bcl6,Irf2,Irf4,Irf9,Nfkb1,Stat5a,Stat6
GO:0002286	**T cell activation involved in immune response**	6	4.21E-06	Bcl6,Irf2,Irf4,Nfkb1,Stat5a,Stat6
GO:0002699	**positive regulation of immune effector process**	6	4.62E-06	Cebpg,Fos,Hsf1,Irf4,Junb,Stat6
GO:0002460	**Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains**	7	1.31E-04	Bcl6,Cebpb,Irf4,Irf9,Nfkb1,Pou2af1,Stat6
GO:0010758	**Regulation of macrophage chemotaxis**	3	4.10E-04	Irf3,Irf7,Nfic
GO:0048246	**Macrophage chemotaxis**	3	0.001409857	Irf3,Irf7,Nfic

Table S6. Antibodies used for FACS and confocal microscopy

Antibody	Company	Clone/Cat. no.
CD45	Invitrogen	30-F11
CD11b	Biolegend	M1/70
CD11c	Biolegend	N418
CD169	eBioscience	Ser-4
CD8α	eBioscience	53-6.7
NK1.1	Biolegend	PK136
CD3	Biolegend	145-2C11
B220	eBioscience	RA3-6B2
IgD	Biolegend	11-26c.2a
MHCII	Biolegend	AF6-120.1
F4/80	Biolegend	BM8
Ly6C	Biolegend	HK1.4
Ly6G	Biolegend	18A
CCR2	R&D Systems	FAB5538P
CD16/32	Biolegend	93
UV Live-Dead	Invitrogen	A-10168
IFN-γ	BD Bioscience	XMG1.2
Listeria	Fisher Scientific	DF2302-50-0

Supplementary Movie Legends:

Movie S1. *Listeria* is captured by CD169^{+} macrophages early after infection. 3D reconstruction of the combined z stacks image shown in Fig.1B. The isosurfaced masking of the cellular volume using the CD169 fluorescence shows that the bacteria is inside CD169+ macrophage at 3 hpi. (Lm; green, CD169; Blue)

Movie S2. *Listeria* is captured by CD169^{+} macrophages early after infection. Another example of isosurfaced masking of the cellular volume using the CD169 fluorescence showing the bacteria inside CD169+ macrophage at 3 hpi. (Lm; green, CD169; Blue, CD11c; red)

Movie S3. CD169^{+} macrophages transfer bacteria to CD8α^{+} DCs. 3D reconstruction of the combined z stacks image shown in Fig.3C depicting the apparent transfer of bacteria by the CD169+ macrophages to CD8α+ DC. Note that the CD8α+ DC interacts closely with the infected CD169+ macrophage. (Lm; green, CD169; Blue, CD11c; red)