Supplementary Information

Tissue-wide immunity against *Leishmania* through collective production of nitric oxide

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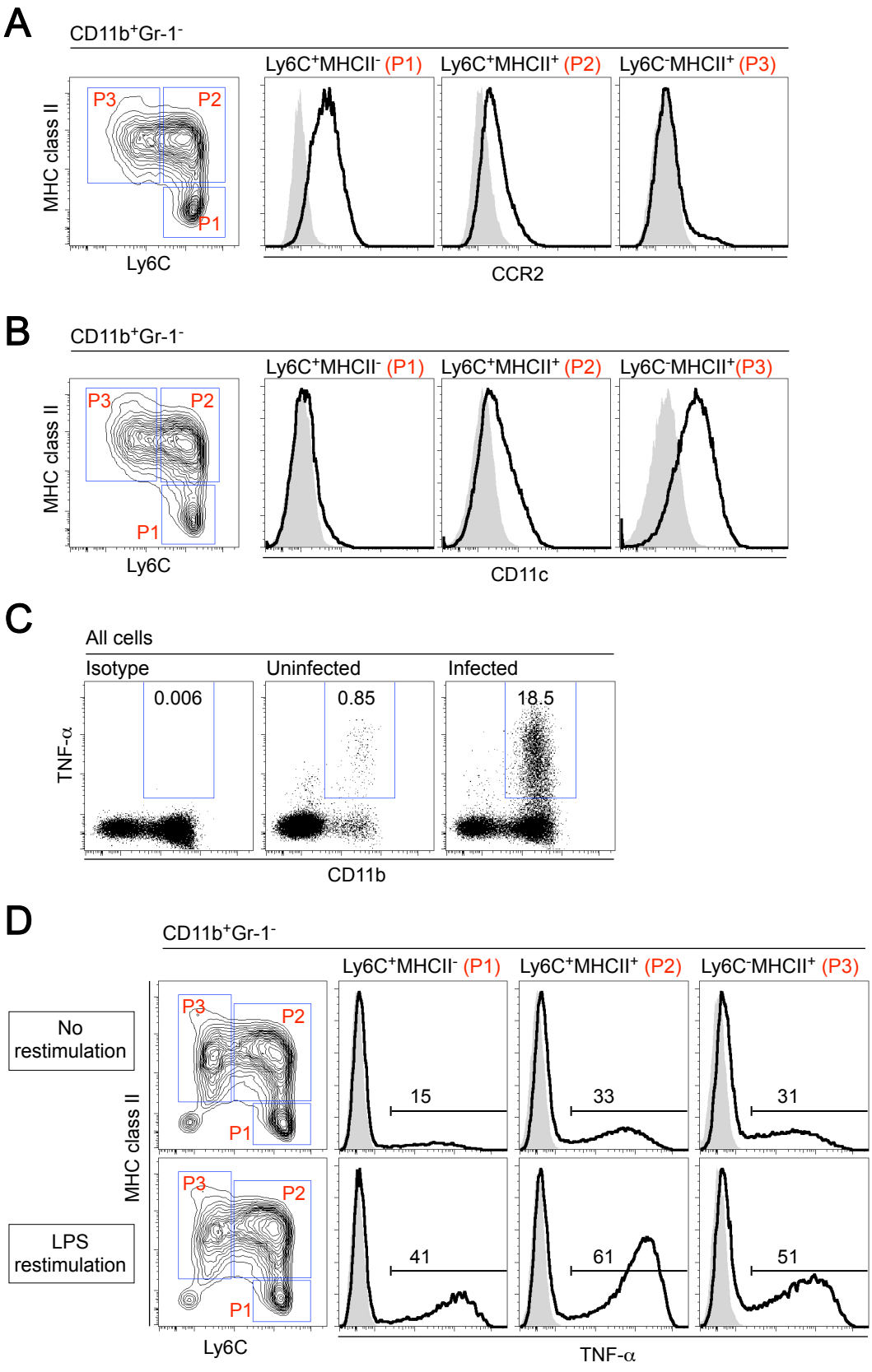
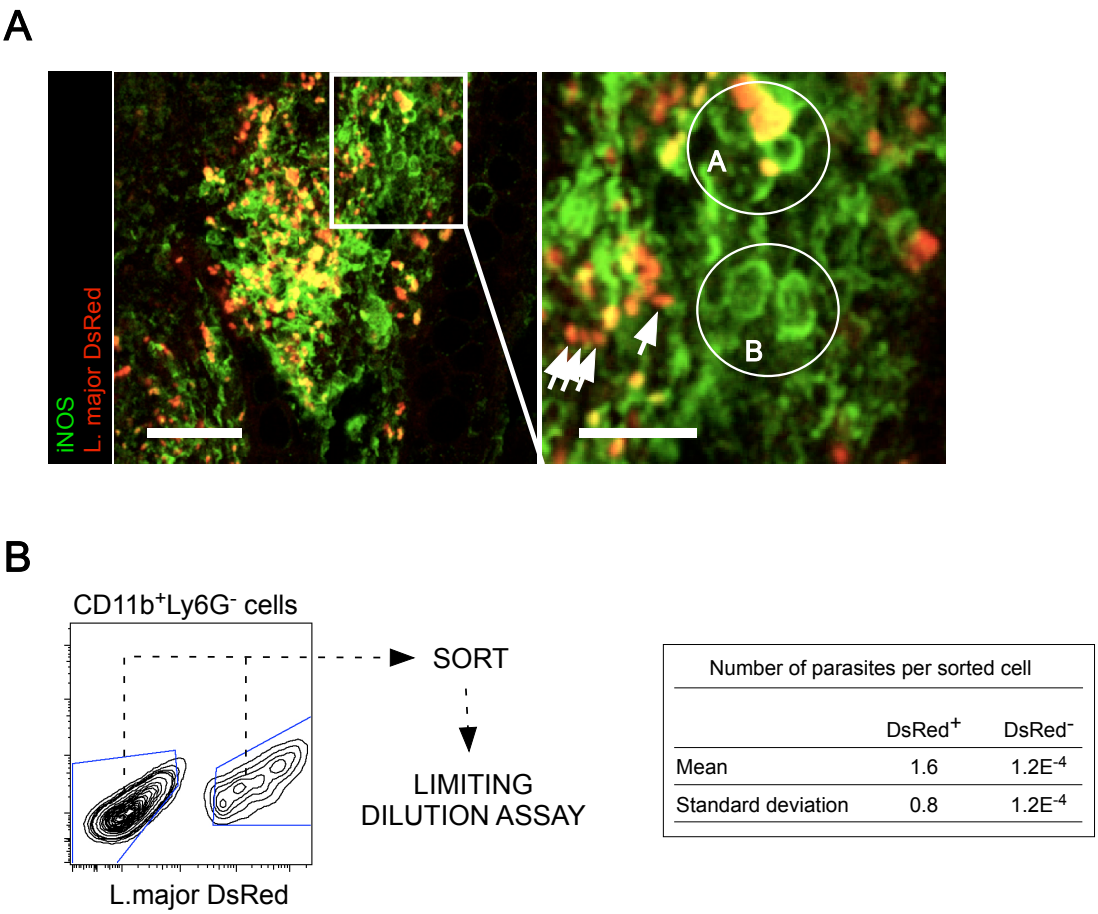
Supplementary Figures

Figure S1. Phenotypic characterization of mononuclear phagocytes at the site of *L. major* infection. (A-B) C57BL/6 mice were infected with DsRed-expressing *L. major* parasites in the ear dermis. Infected ear tissues were analyzed two weeks later by flow cytometry. Representative flow cytometry plots showing CCR2 **(A)** and CD11c **(B)** expression on cells falling into the indicated region. Gray solid curves indicate control isotype staining. **(C-D)** Ear tissue cell suspensions from mice infected for two weeks of left uninfected were cultured with or without LPS for 4 hr in the presence of BFA, stained for intracellular TNF-α (control isotype staining was performed on infected ear tissue) and analyzed by flow cytometry. **(C)** Representative flow cytometry profiles corresponding to total cells cultured without LPS stimulation. **(D)** TNF-α expression in the various mPhagocyte subsets recovered from infected ears. Data are representative of two independent experiments.


A
CD11b+Gr-1-
Ly6C+MHCII- (P1)
Ly6C+MHCII+ (P2)
Ly6C-MHCII+ (P3)
MHC class II
P3
P2
P1
Ly6C
CCR2
B
CD11b+Gr-1-
Ly6C+MHCII- (P1)
Ly6C+MHCII+ (P2)
Ly6C-MHCII+(P3)
MHC class II
P3
P2
P1
Ly6C
CD11c
C
All cells
Isotype
Uninfected
Infected
0.006
0.85
18.5
TNF-α
CD11b
D
CD11b+Gr-1-
Ly6C+MHCII- (P1)
Ly6C+MHCII+ (P2)
Ly6C-MHCII+ (P3)
No restimulation
LPS restimulation
MHC class II
P3
P2
P1
15
33
31
41
61
51
Ly6C
TNF-α



Number of parasites per sorted cell		
	DsRed^{+}	DsRed^{-}
Mean	1.6	1.2E^{-4}
Standard deviation	0.8	1.2E^{-4}

Figure S2. Cell infection by *L. major* parasites is not required for iNOS induction. (A) C57BL/6 mice were infected with DsRed-expressing *L. major* parasites in the ear dermis. Two weeks later, fixed cryosections of infected ear tissues were analyzed by confocal immunofluorescence microscopy. The right panel shows an enlargement of the area delimited by a white rectangle on the left panel. In the right panel, area A shows infected iNOS^{+} cells ; area B shows uninfected iNOS^{+} cells ; arrows indicate individual parasites. Scale bars, 50 μm (left panel) and 20 μm (right panel). **(B)** C57BL/6 mice were infected with DsRed-expressing *L. major* parasites in the ear dermis. Two weeks later, infected ears were analyzed by flow cytometry and CD11b^{+}Ly6G^{-} cells were sorted according to their *L. major* DsRed fluorescence. Parasite counts in DsRed^{+} and DsRed^{-} cells were determined by a limiting-dilution assay and normalized to sorted cell numbers.

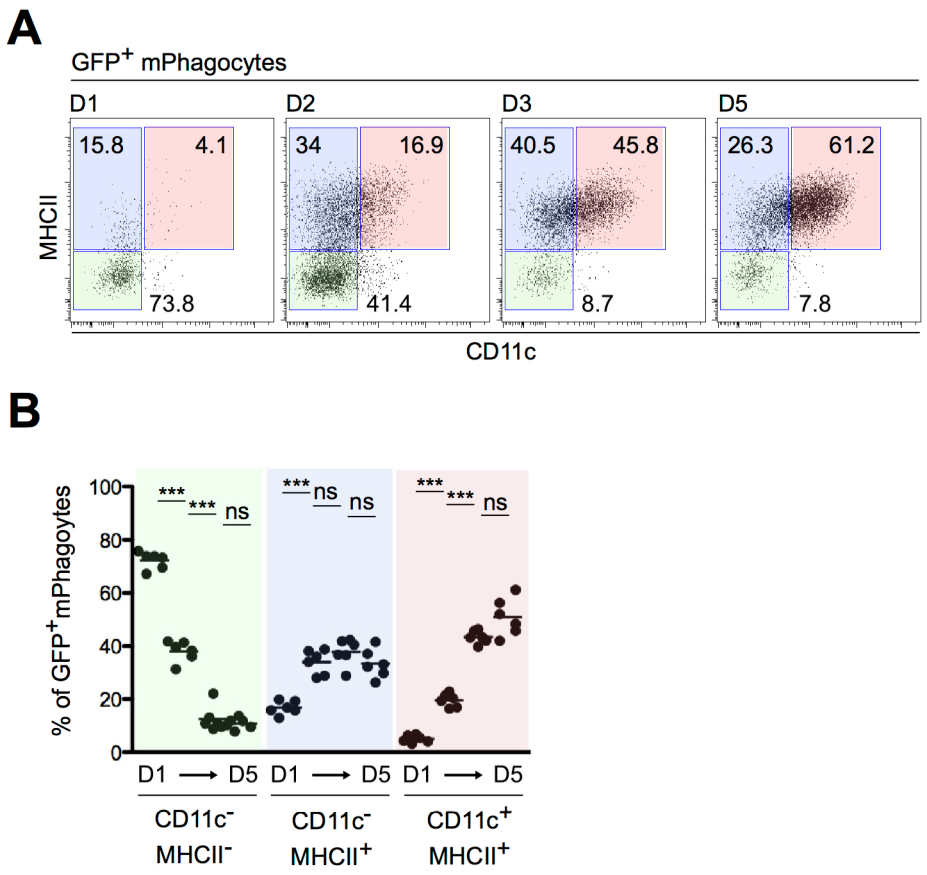


Figure S3. Tracking the fate of recruited phagocytes at the site of infection: MHC class II and CD11c expression. C57BL/6 mice were infected with DsRed-expressing *L. major* parasites in the ear dermis. Two weeks later, GFP-expressing BMCs were transferred to infected recipients. Infected ears were analyzed from day 1 to day 5 post injection by flow cytometry. **(A)** Representative flow cytometry plots of infected ear tissues. **(B)** Plots correspond to cell population frequencies measured by flow cytometry. Each dot represents an individual ear and horizontal lines represent average values. Significance is denoted by asterisks; ns, not significant. Data are representative of two independent experiments.

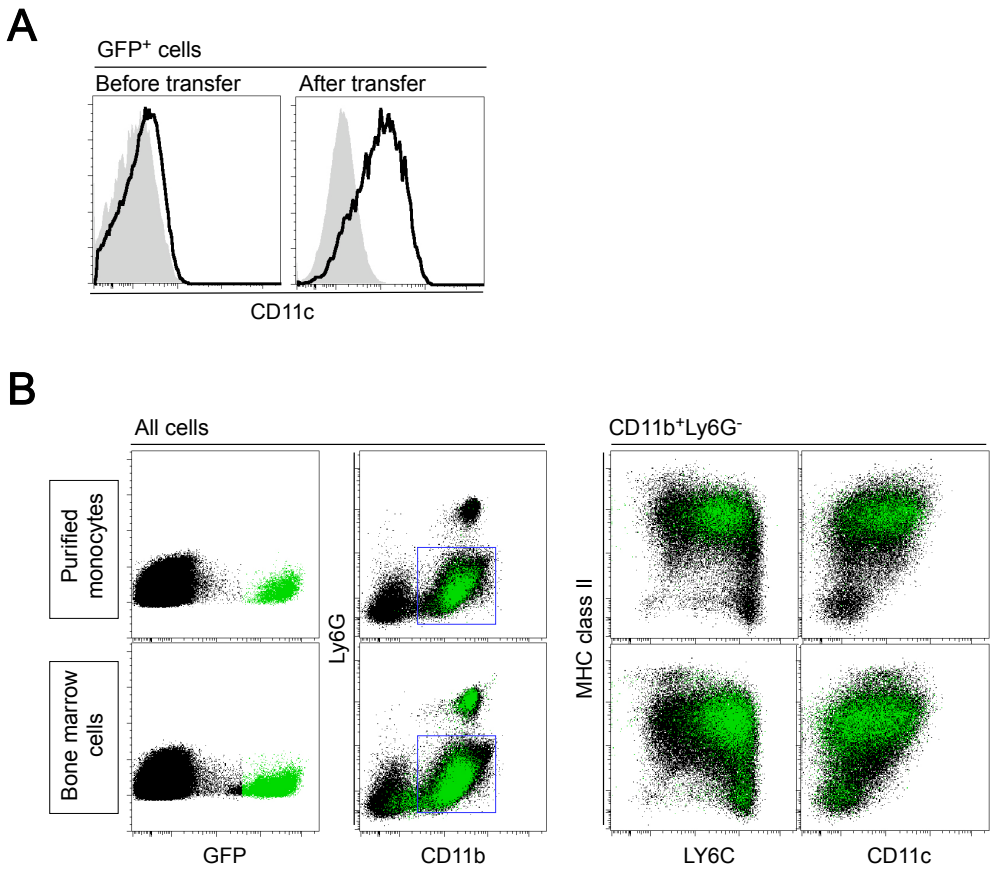


Figure S4. Tracking the fate of recruited phagocytes after transfer of purified monocytes.

C57BL/6 mice were infected with DsRed-expressing *L. major* parasites in the ear dermis. Two weeks later, 2-3.10^6 purified GFP-expressing monocytes or 50.10^6 GFP-expressing BMCs were transferred to infected recipients. Infected ears were analyzed at day 4 post injection by flow cytometry. (A) Representative histograms showing CD11c expression on purified GFP^+ monocytes (before transfer) and on GFP^+ (monocyte-derived) cells recovered from infected ears (after transfer). (B) Phenotypic characterization of GFP^+ cells in infected ears after transfer of purified GFP^+ monocytes or total GFP^+ bone marrow cells. Representative flow cytometry profiles corresponding to GFP^+ cells (green) and GFP^- cells (black). Data are representative of two independent experiments.

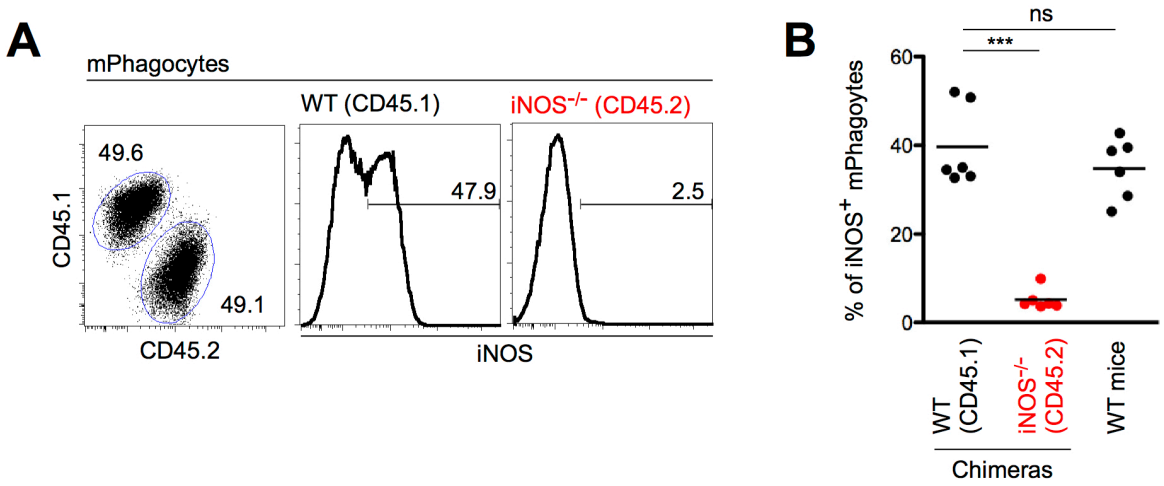


Figure S5. iNOS expression in mixed bone marrow chimeras. Lethally irradiated C57BL/6 WT mice were reconstituted with a mixture of 50% CD45.1 WT and 50% CD45.2 iNOS$^{-/-}$ BMCs. Mixed BM chimeras were infected with DsRed-expressing *L. major* parasites in the ear dermis and two weeks later, infected ear tissues were stained for intracellular iNOS and analyzed by flow cytometry. **(A)** Representative flow cytometry plots of infected ear tissues from mixed BM chimeras. **(B)** Frequency of iNOS^{+} cells among CD45.1 WT and CD45.2 iNOS$^{-/-}$ mPhagocytes in the ear of BM chimeras and among mPhagocytes in the ear of WT mice measured by flow cytometry. Each dot represents an individual ear and horizontal lines represent average values. Significance is denoted by asterisks; ns, not significant. Data are representative of two independent experiments.

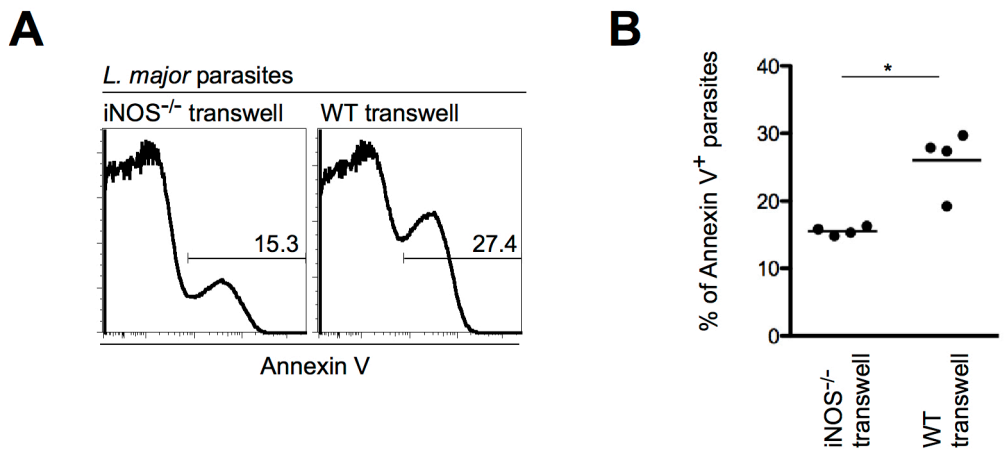


Figure S6. Nitric oxide released from activated macrophages affects the viability of spatially separated free *L. major* parasites. Peritoneal macrophages from WT and iNOS$^{-/-}$ mice were put in culture and activated with LPS and IFN-γ. *L. major* parasites (10^7 promastigotes) were added on top in transwell inserts. Two days later, *L. major* parasites were harvested, stained with Annexin V and analyzed by flow cytometry. **(A)** Representative histograms of Annexin V fluorescence on *L. major* parasites cultured in the presence of either WT or iNOS$^{-/-}$ macrophages. **(B)** Frequency of Annexin V^{+} parasites cultured in the presence of either WT or iNOS$^{-/-}$ macrophages. Each dot represents an individual well and horizontal lines represent average values. Significance is denoted by asterisks.

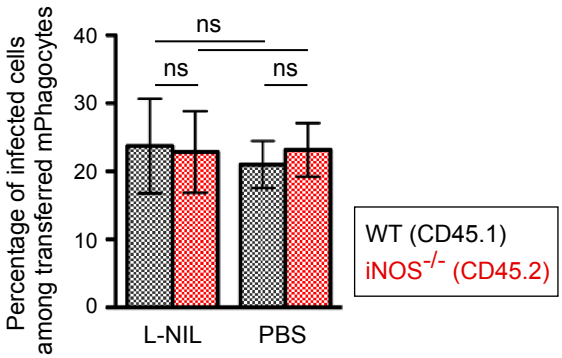


Figure S7. iNOS competent cells do not exhibit any antimicrobial activity in an iNOS deficient environment. iNOS$^{-/-}$ mice were infected and 2 weeks later were injected with a CFSE labeled mixture of 50% CD45.1 WT and 50% CD45.2 iNOS$^{-/-}$ BMCs. Half of infected mice received daily injections of L-NIL during the three following days and ear tissues were then harvested and analyzed by flow cytometry. Graph shows the frequency of infected cells in labeled mPhagocytes obtained by flow cytometry analysis. Means $\pm$ SEM are shown. ns, not significant.

Legends for movies

Movie 1. Phagocytes recruited in the infected ear get infected by *L. major* parasites. C57BL/6 mice were infected with *L. major* and two weeks later, GFP-BMCs isolated from GFP-expressing *Rag2*$^{-/-}$ mice were transferred into infected recipients. Three days post infection, mice were anaesthetized and intravital microscopy was performed on infected ears. Movie shows phagocyte (green) invasion by a *L. major* parasite (red). The invading parasite is indicated by a white cirle.

Movie 2. Visualizing parasite killing in activated WT macrophages. Peritoneal macrophages from WT mice were infected with *L. major* parasites (red) and activated two days later. After 10 h, time-lapse microscopy was performed for up to 48 h.

Movie 3. Lack of parasite killing in activated iNOS-/- macrophages. Peritoneal macrophages from iNOS$^{-/-}$ mice were infected with *L. major* parasites (red) and activated two days later. After 10 h, time-lapse microscopy was performed for up to 48 h.

Movie 4. Parasite killing *in trans*. Peritoneal macrophages from WT GFP^{+} (green) and iNOS$^{-/-}$ (unlabeled) mice were mixed in the same well, infected with *L. major* parasites (red) and activated two days later. After 10 h, time-lapse microscopy was performed for up to 48 h. Note that parasite killing occurs simultaneously in iNOS$^{-/-}$ and WT macrophages.