Supporting Information

Duque-Correa et al. 10.1073/pnas.1408839111

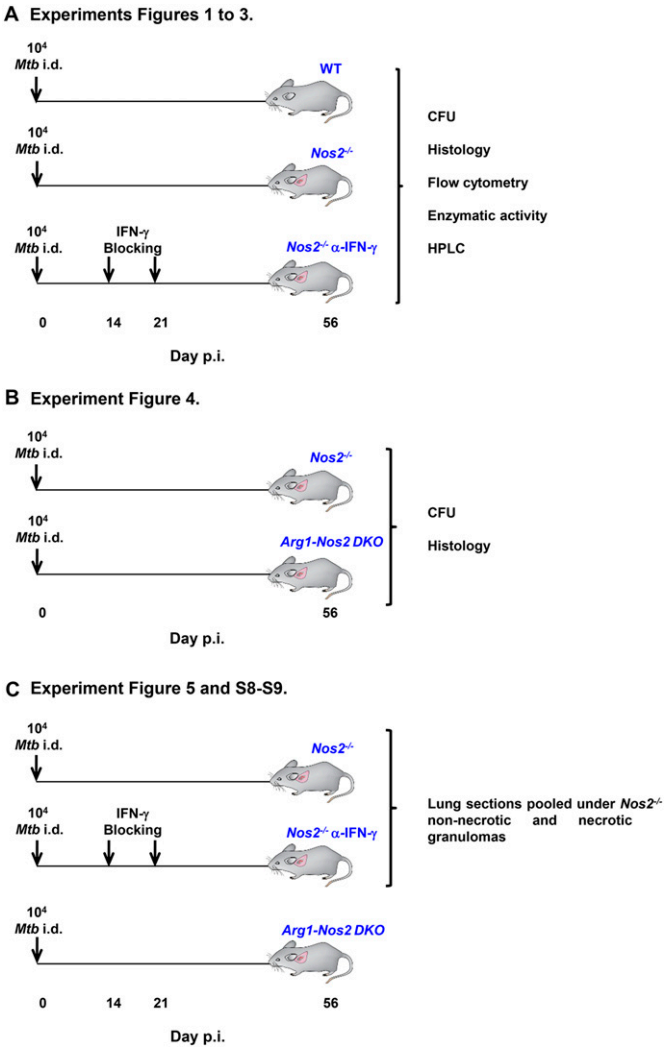

Fig. S1. Experimental strategies used throughout the study. (*A*) The granuloma model of nitric oxide synthase-2–deficient (*Nos2*$^{-/-}$) mice dermally infected with *Mycobacterium tuberculosis* (*Mtb*): WT and *Nos2*$^{-/-}$ mice were infected intradermally with 10^4 *Mtb*. At days 14 and 21 postinfection (p.i.), *Nos2*$^{-/-}$ mice received 500 μg anti–IFN-γ i.p. or remained untreated. Mice were killed at day 56 p.i., and tissues were used for further analysis. (*B*) *Nos2*$^{-/-}$ and arginase-1 (*Arg1*)-*Nos2* double knockout (DKO) mice were infected intradermally with 10^4 *Mtb*. Mice were killed at day 56 p.i., and tissues were used to assess bacterial burden and for histological analysis. (*C*) *Nos2*$^{-/-}$ and *Arg1-Nos2* DKO mice were infected intradermally with 10^4 *Mtb*. At days 14 and 21 p.i. *Nos2*$^{-/-}$ mice received 500 μg anti–IFN-γ i.p. or remained untreated. Mice were killed at day 56 p.i., and tissues were used for histological analysis. To increase the number of nonnecrotic and necrotic granulomas from *Nos2*$^{-/-}$ mice, lung sections from *Nos2*$^{-/-}$ and IFN-γ–blocked *Nos2*$^{-/-}$ mice were pooled.

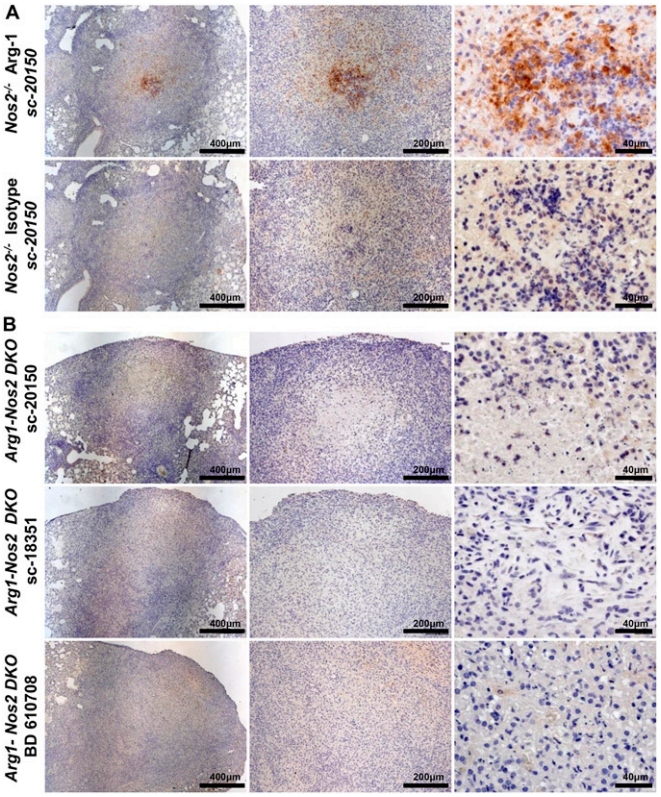

Fig. S2. (*A*) Immunohistochemical (IHC) staining of representative necrotic granuloma from infected IFN-γ–blocked *Nos2*$^{-/-}$ mice at day 56 p.i. using anti-Arg1 (sc-20150; Santa Cruz) and corresponding isotype. (*B*) IHC staining of representative necrotic granuloma from infected *Arg1-Nos2* DKO mice at day 56 p.i. using anti-Arg1 from Santa Cruz (sc-20150 and sc-18351) and from BD (610708).

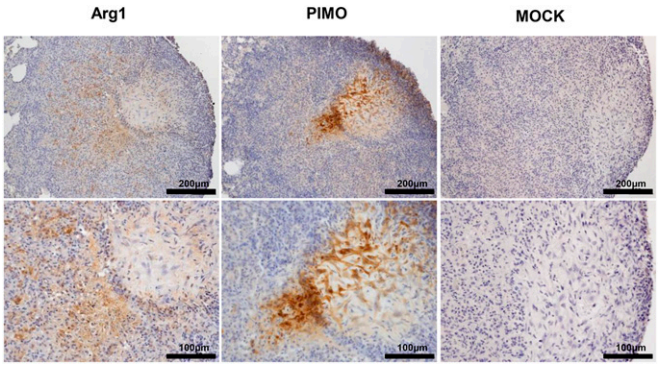

Fig. S3. Arg1^{+} cells surround the necrotic core of caseous granulomas where hypoxic cells are localized. At day 56 p.i., infected mice received the tissue hypoxia marker pimonidazole (PIMO) and were killed 2 h later. (Mock: one animal was left untreated.) Lung sections were stained with anti-Arg1 mAb and anti-PIMO mAb. Images show a representative necrotic tuberculosis (TB) lung granuloma from an infected IFN-γ–blocked *Nos2*$^{-/-}$ mouse.

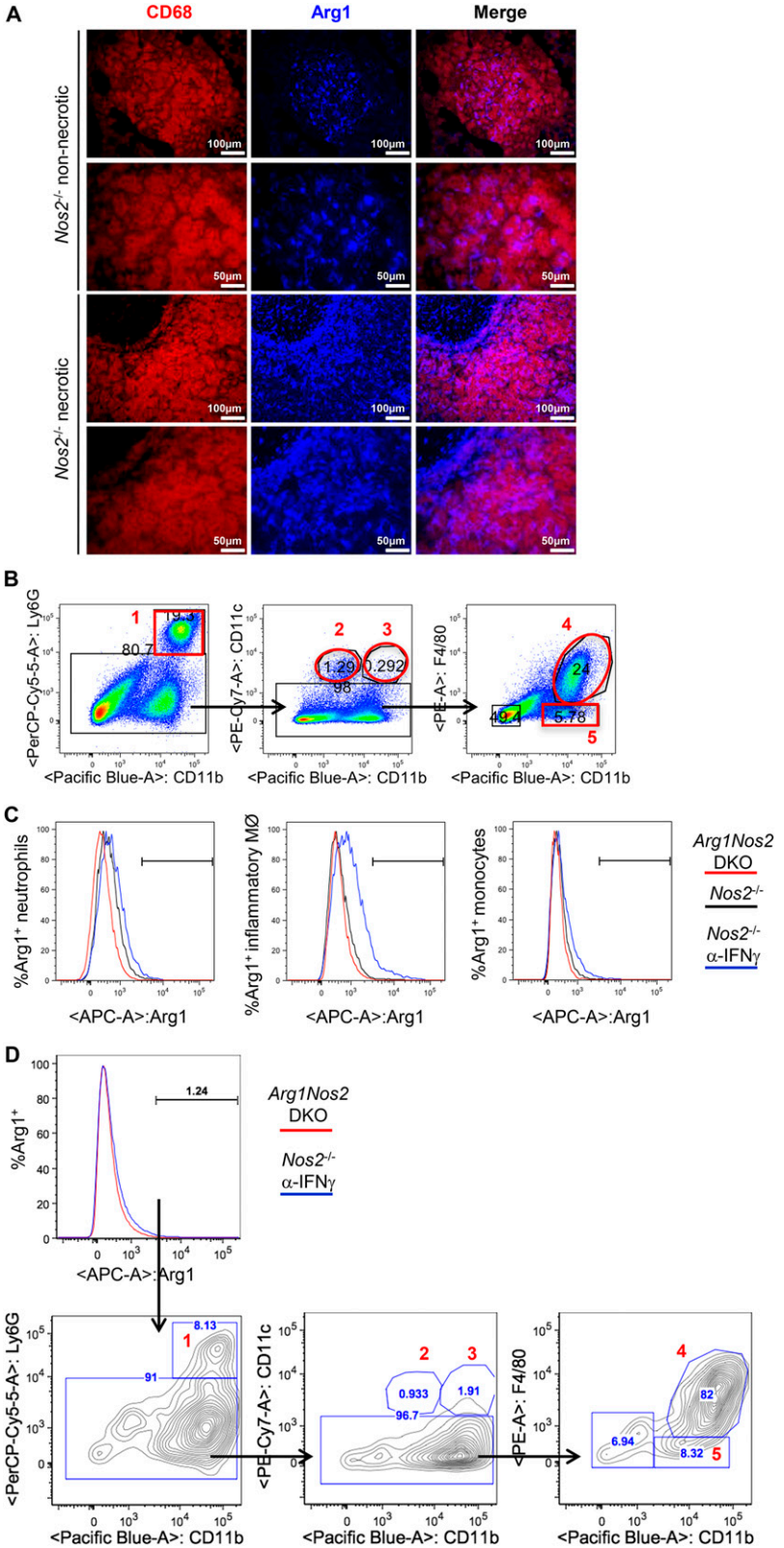

Fig. S4. Macrophages are the main cell type expressing Arg1 in lungs from infected *Nos2*$^{-/-}$ mice. (*A*) Representative nonnecrotic and necrotic TB lung granulomas from infected *Nos2*$^{-/-}$ mice at day 56 p.i. stained with anti-CD68 (red) and anti-Arg1 (blue). Staining shows colocalization of Arg1^{+} cells and CD68^{+} cells indicating that Arg1 is expressed in macrophages in TB lung granulomas. (*B*) Gating strategy used to characterize phagocytic cell populations of the lungs of infected *Nos2*$^{-/-}$ and IFN-γ–blocked *Nos2*$^{-/-}$ mice. Populations: 1, neutrophils; 2, alveolar macrophages; 3, dendritic cells; 4, inflammatory macrophages; 5, monocytes. (*C*) Histograms representative of Arg1 expression in neutrophils, inflammatory macrophages, and monocytes. The red line depicts *Arg1-Nos2* DKO mice, the black line represents *Nos2*$^{-/-}$ mice, and the blue line represents IFN-γ–blocked *Nos2*$^{-/-}$ mice. (*D*) Alternative gating strategy for characterization of Arg1-expressing cell populations in the lungs of infected *Nos2*$^{-/-}$ and IFN-γ–blocked *Nos2*$^{-/-}$ mice. Arg1^{+} cells are gated in the leukocyte population, and then the populations expressing Arg1 are identified as in *B*.

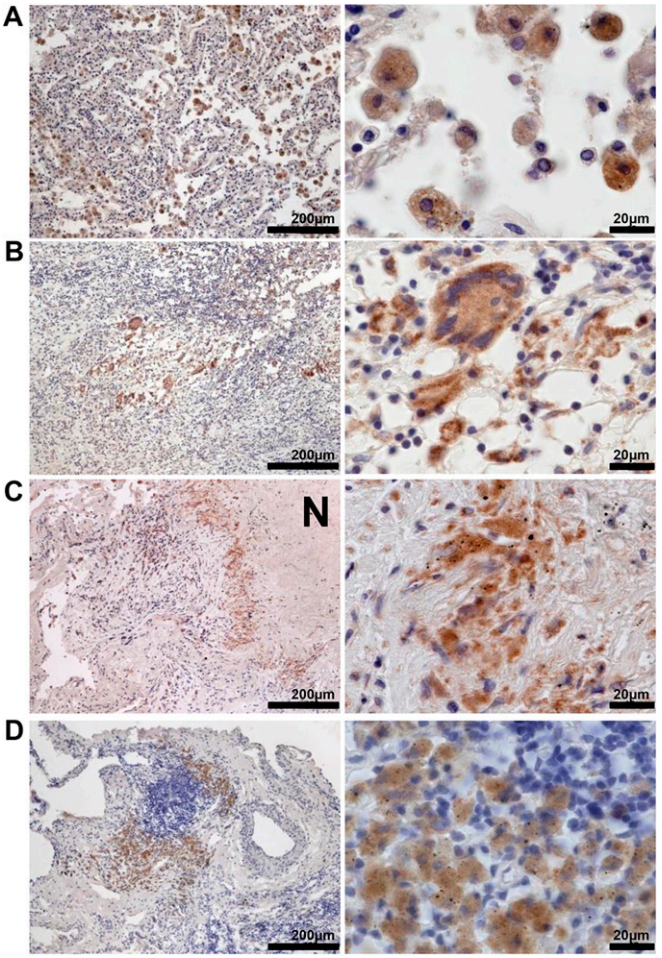

Fig. S5. Arg1 is expressed in human lung tissue of TB patients. Human lung samples taken from patients with TB stained with anti-Arg1 mAb. (*A* and *B*) Pericavity lung tissue. (*C*) Cavity wall lung tissue. (*D*) Distal lung tissue. N, necrotic.

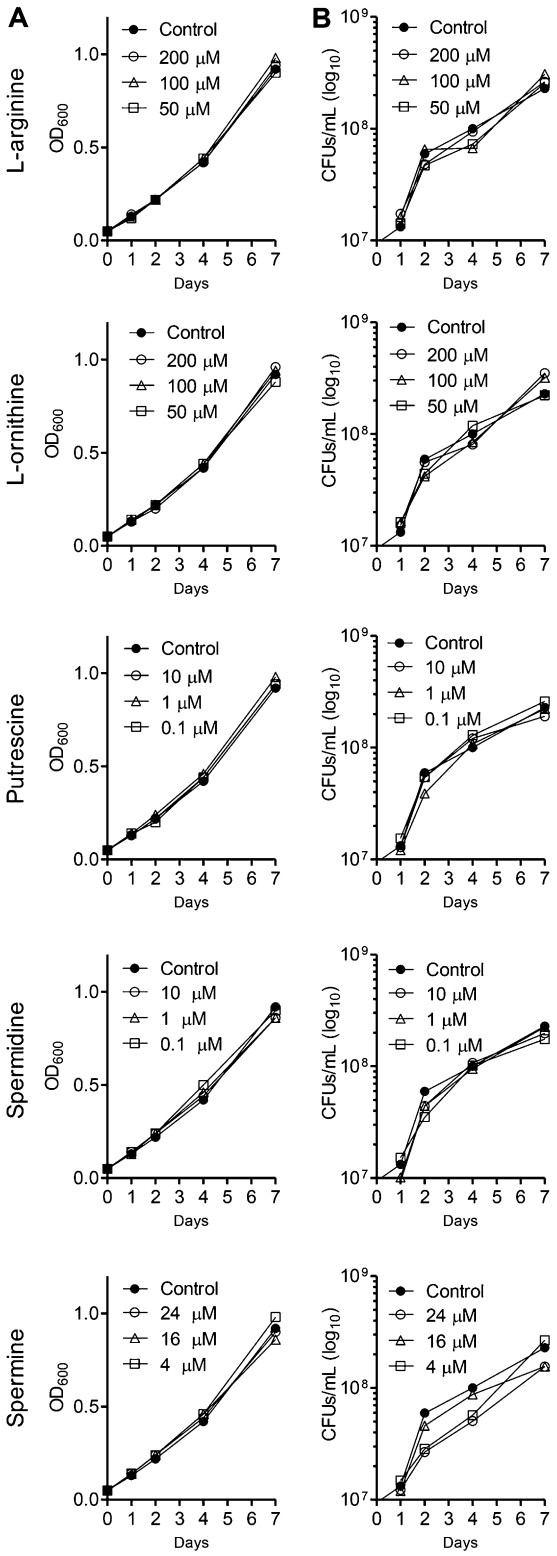

Fig. S6. L-arginine–derived metabolites do not affect *Mtb* growth in culture. *Mtb* H37Rv was cultured in Sauton's minimal medium supplemented with L-arginine, L-ornithine, putrescine, spermidine, and spermine. At days 1, 2, 4, and 7 bacterial growth was evaluated by measurement of OD_{600} (*A*) and by enumerating cfus (*B*). Data are representative of two independent experiments.

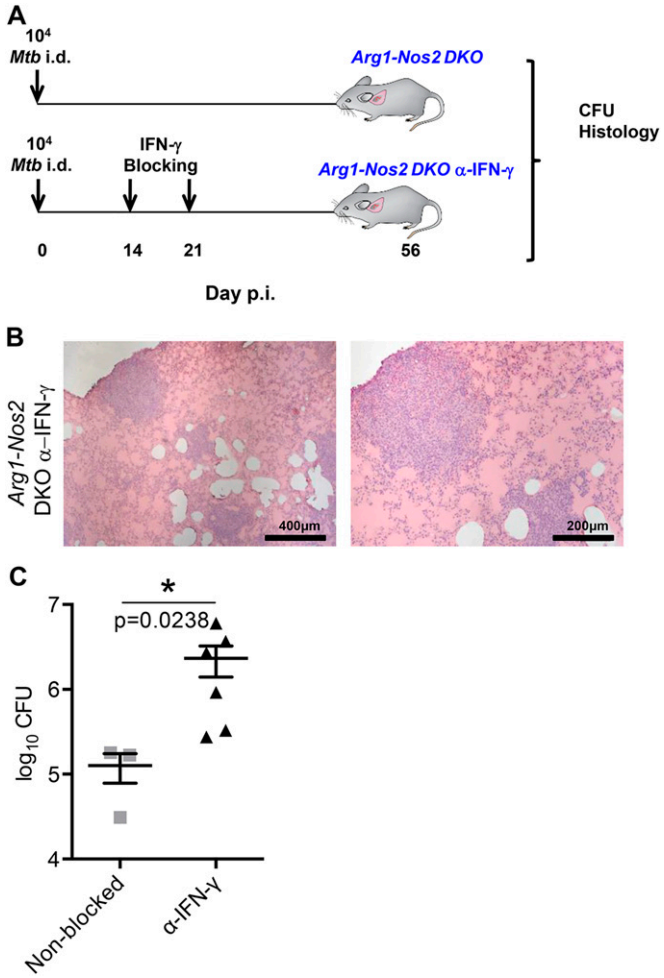

Fig. S7. Blocking of IFN-γ in infected *Arg1-Nos2* DKO mice exacerbates *Mtb* growth and pathology in TB lung granulomas. (*A*) Experimental strategy to evaluate the effect of IFN-γ blocking in infected *Arg1-Nos2* DKO mice: *Arg1-Nos2* DKO mice were infected intradermally with 10^4 *Mtb*. At days 14 and 21 p.i., mice received 500 μg anti–IFN-γ i.p. or remained untreated. Mice were killed at day 56 p.i., and tissues were used for bacterial burden and histological analysis. (*B*) H&E staining of lung tissue from infected IFN-γ–blocked *Arg1-Nos2* DKO mice at day 56 p.i. (*C*) Lung cfus from infected, nonblocked *Arg1-Nos2* DKO ($n = 3$) and IFN-γ–blocked *Arg1-Nos2* DKO ($n = 6$) mice at day 56 p.i. Data show the median and interquartile range. Significance was assessed by the Mann–Whitney test.

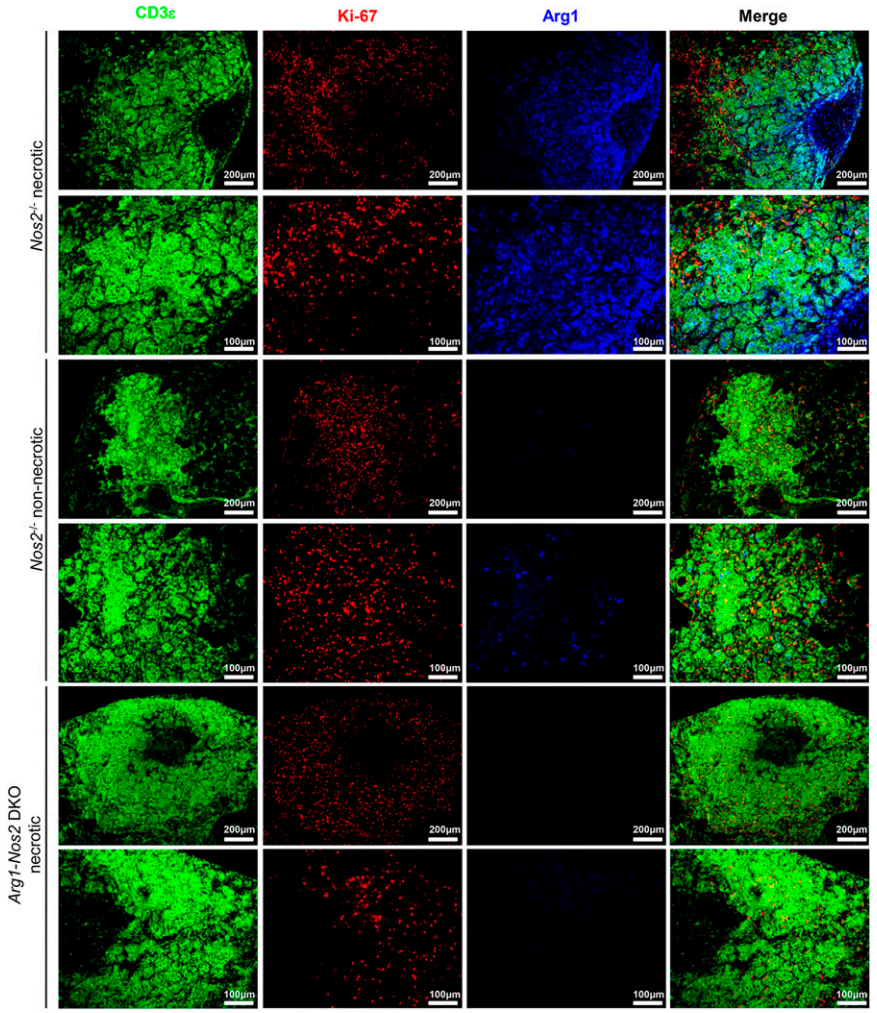

Fig. S8. Proximity of Arg1^{+} cells affects T-cell proliferation in microenvironments within granulomas. Arg1, Ki-67, and CD3ε immunofluorescence staining of representative nonnecrotic and necrotic TB lung granulomas from infected *Nos2*$^{-/-}$ and *Arg1-Nos2* DKO mice at day 56 p.i.

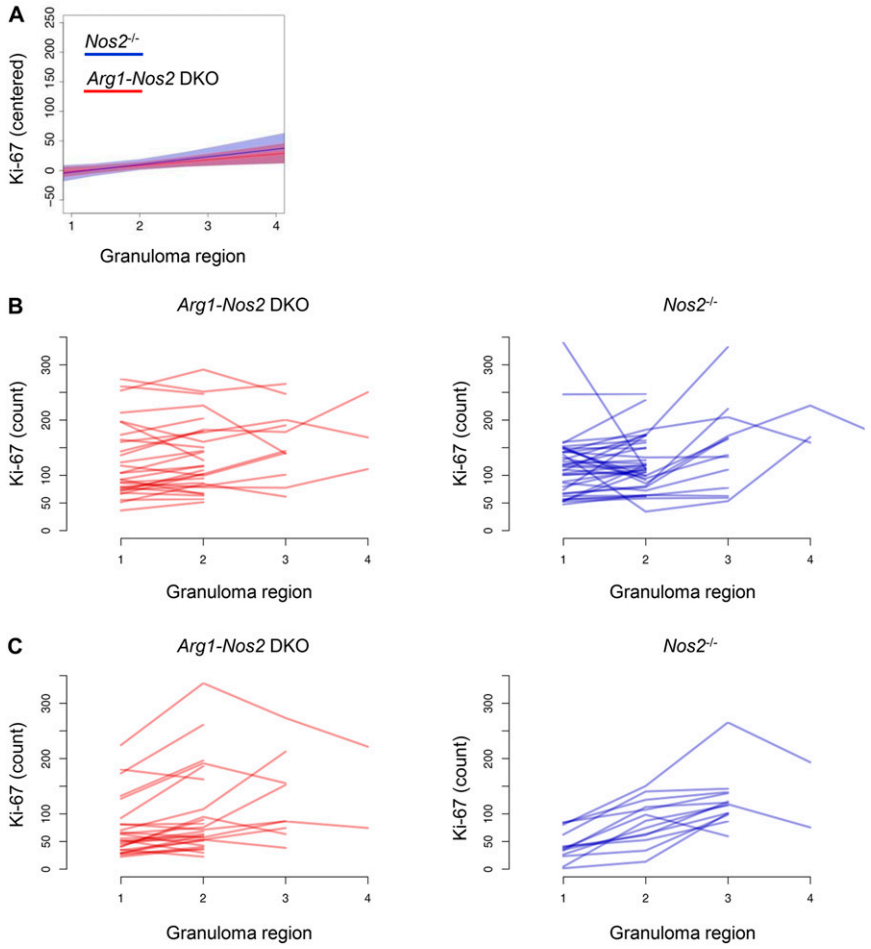

Fig. S9. The gradients of the numbers of Ki-67$^+$ cells in nonnecrotic granulomas from infected Nos2$^{-/-}$ and Arg1-Nos2 DKO mice at day 56 p.i. are not different. (*A*) Linear regression of the gradient of number of Ki-67$^+$ cells in nonnecrotic granulomas from infected *Nos2*$^{-/-}$ and IFN-γ–blocked *Nos2*$^{-/-}$ mice (blue lines) and from *Arg1-Nos2* DKO mice (red lines) at day 56 p.i. Lines correspond to linear fits of centered Ki-67$^+$ cell values on separate granuloma regions for each of the two subgroups. Shaded areas show corresponding 95% confidence bands. (*B* and *C*) Counts of Ki-67$^+$ cells through granuloma regions 1–4 of individual nonnecrotic (*B*) and necrotic (*C*) granulomas from infected *Nos2*$^{-/-}$ and *Arg1-Nos2* DKO mice.