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Supplemental Information

CD8α$^+$ Dendritic Cells Are the Critical Source of Interleukin-12 that Controls Acute Infection by *Toxoplasma gondii* Tachyzoites

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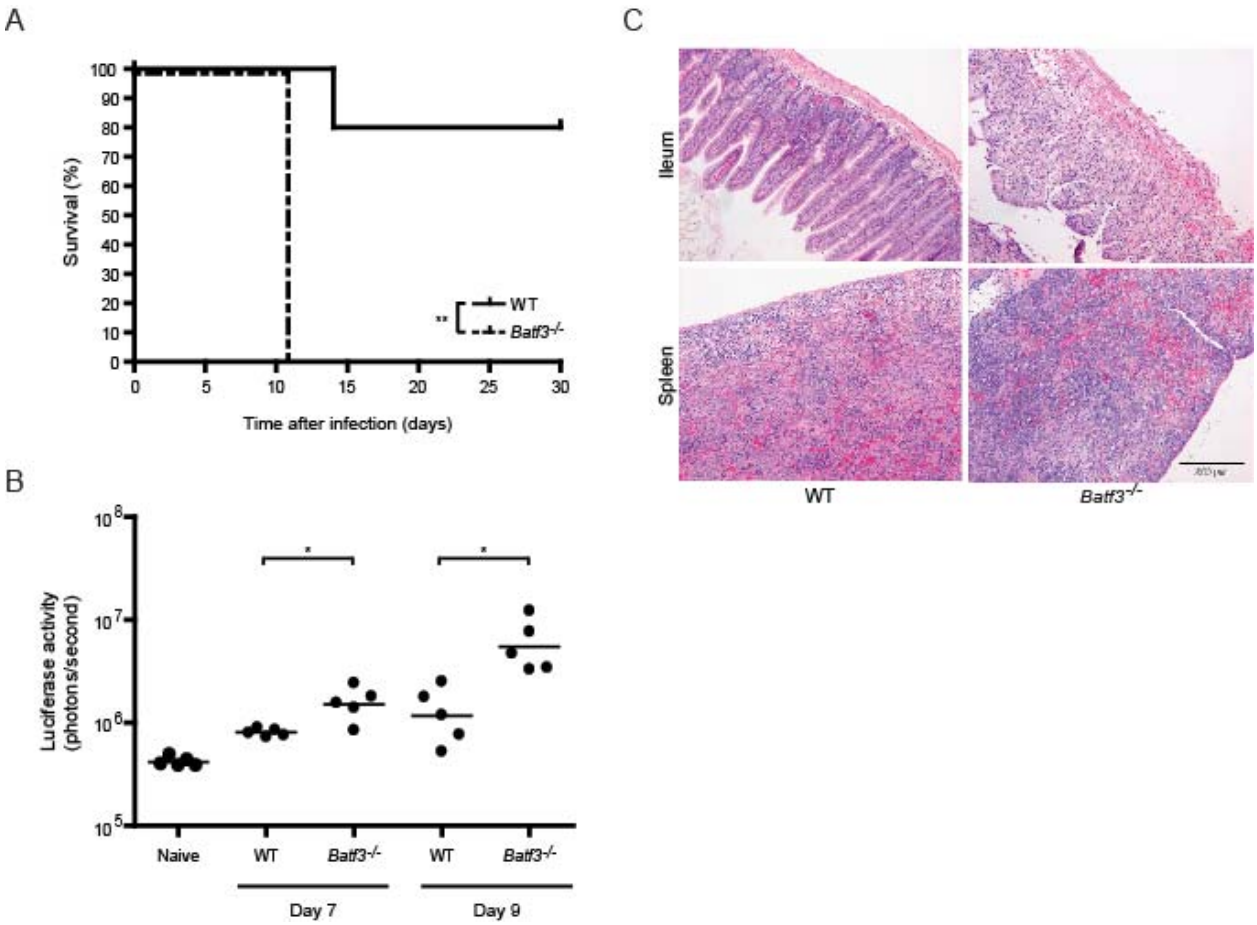

Figure S1, related to Figure 1. *Batf3*$^{-/-}$ mice are highly susceptible to oral infection with *T. gondii* cysts and cannot control parasite replication. Mice were infected with 5 ME49-FLuc tissue cysts by oral gavage, and monitored for survival (A) and parasite burden (B). (A) BALB/c wild-type (solid line) and *Batf3*$^{-/-}$ (dashed line) mice were monitored for survival over the course of the experiment (n=5, representative of 2 independent experiments). (B) Infected wild-type and *Batf3*$^{-/-}$ mice were monitored by whole body *in vivo* bioluminescence imaging on day 7 and 9 after infection (n=5, representative of 2 independent experiments). Horizontal lines represent the

geometric mean. (C) 129S6/SvEV wild-type and *Batf3*$^{-/-}$ mice were infected with 20 PRU-FLuc-GFP tissue cysts and sacrificed on day 9 after infection for H&E analysis of the spleen and ileum. *: $0.01<P<0.05$, **: $0.001<P<0.01$.

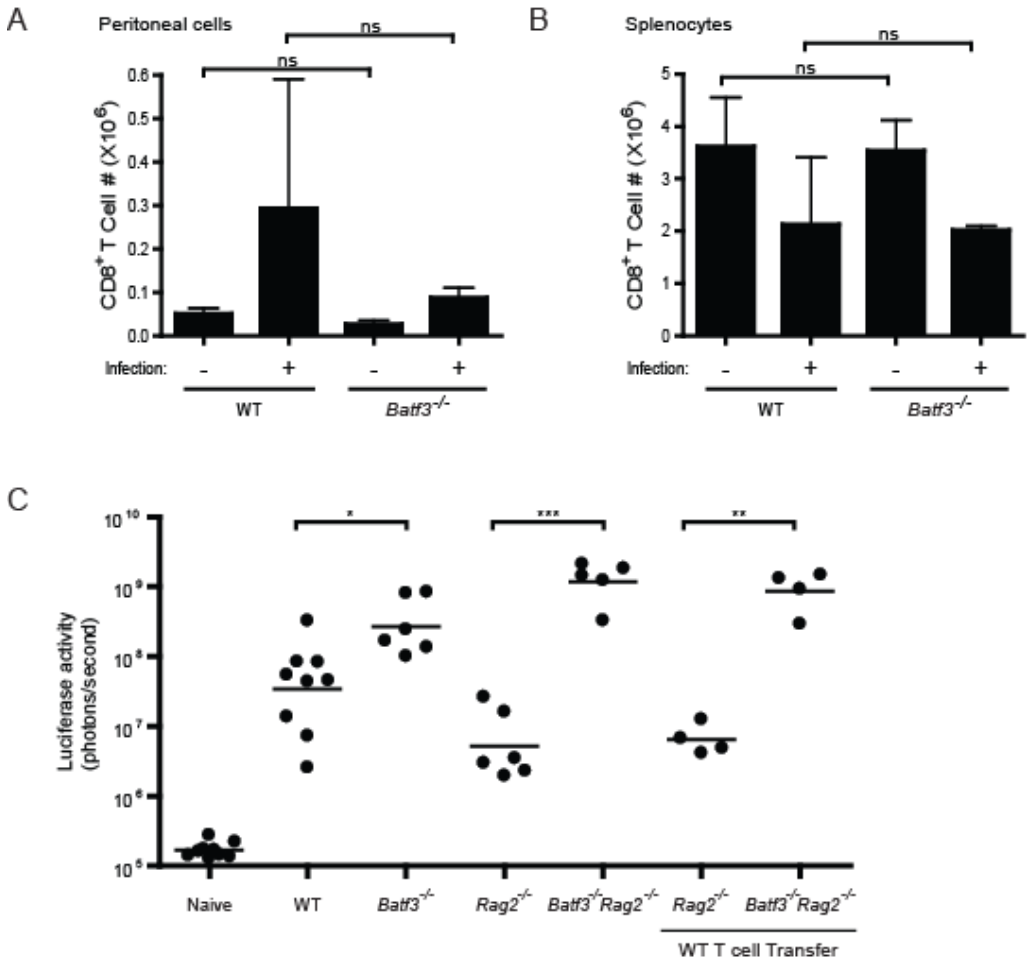

Figure S2, related to Figure 2. CD8$^+$ T cell compartment is normal in *Batf3*$^{-/-}$ mice, and the presence of wild-type T cells in *Batf3*$^{-/-}$ mice does not change their susceptibility to *T. gondii* infection. (A and B) BALB/c wild-type and *Batf3*$^{-/-}$ mice were infected with *T. gondii*, sacrificed on day 8 after infection, and analyzed for total numbers of CD8$^+$ T cells in the spleen (B) and peritoneum (A) (n=3). Data are represented as mean +/- standard deviation. (C) C57BL/6 wild-type, *Batf3*$^{-/-}$, *Rag2*$^{-/-}$, or *Batf3/Rag2*$^{-/-}$ mice were infected with *T. gondii* and parasite burden measured by *in vivo* bioluminescence imaging on day 7 or 8 after infection. In two groups, wild-type T cells were adoptively transferred into the recipient mice one day before infection. Horizontal lines represent the geometric mean. Not significant (ns): P>0.05, *: 0.01<P<0.05, **: 0.001<P<0.01, ***: P<0.001.

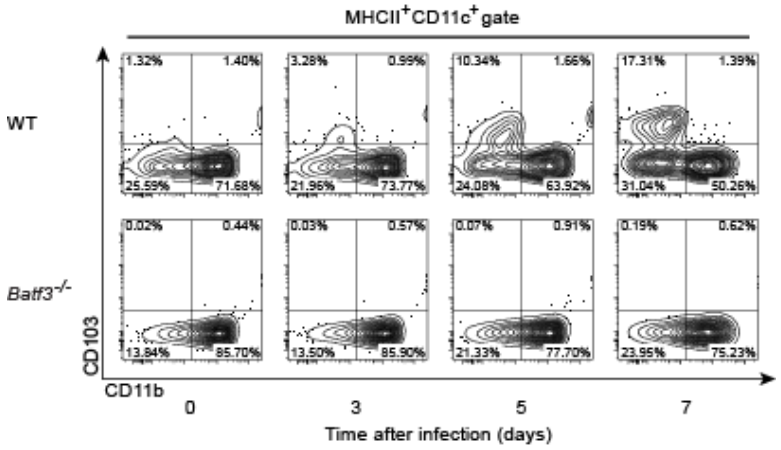

Figure S3, related to Figure 3. CD8α$^+$ DCs expand in response to infection in wild-type mice, while CD11b$^+$ DC percentages remain constant. Representative flow cytometry plots from Figure 3C. Wild-type and *Batf3*$^{-/-}$ 129S6/SvEV mice were infected with *T. gondii*, harvested on days 0, 3, 5 and 7 after infection, and splenocytes analyzed by flow cytometry for DC subsets. Flow cytometry plots gated on MHCII$^+$ CD11c$^+$ DCs (n=3, data representative of 2 independent experiments).

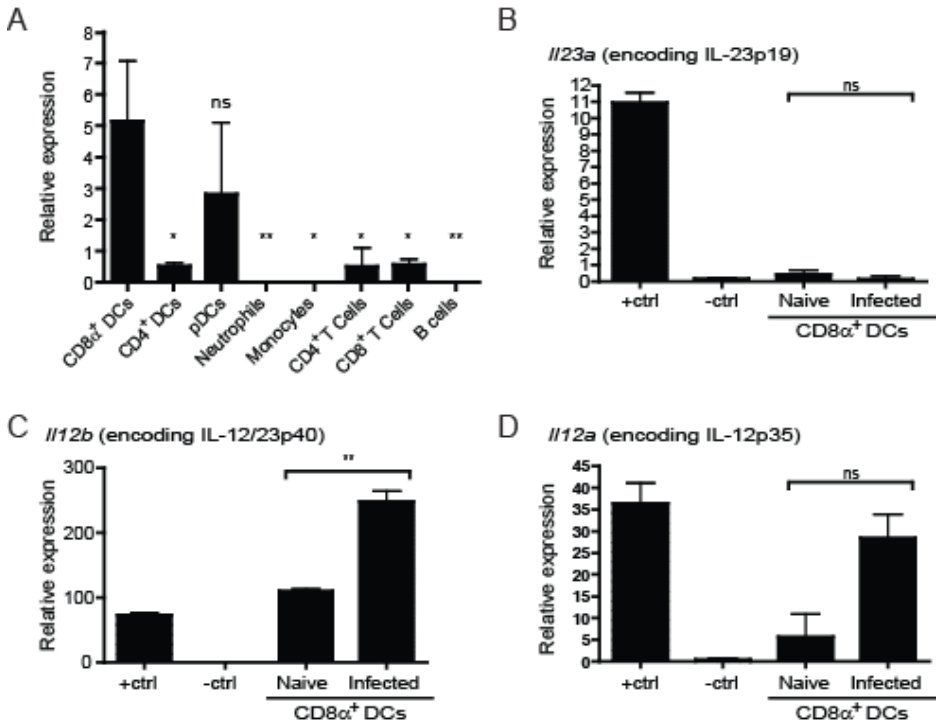

Figure S4, related to Figure 4. CD8α$^+$ DCs express the highest level of the *T. gondii* sensor *Tlr11*, and produce IL-12, but not IL-23 in response to *T. gondii* infection. (A) Listed immune cells were sort-purified from 129S6/SvEV wild-type mice, harvested for RNA, and analyzed for the expression of *Tlr11* by quantitative RT-PCR (n=3). All P-values compare CD8α$^+$ DCs to indicated population. (B-D) CD8α$^+$ DCs were sort-purified from *T. gondii* infected 129S6/SvEV wild-type mice on days 0 and 3 after infection, harvested for RNA, and analyzed for the expression of cytokine chains by qRT-PCR. Two uninfected and three infected spleens were pooled prior to the sort. As positive and negative controls for qRT-PCR, cDNA from total splenic CD11c$^+$ cells, the macrophage cell line J774, and total kidney lysate were also analyzed. *Il23a* (IL-23p19) +ctrl = J774 cDNA, -ctrl = Kidney cDNA; *Il12a* (IL-12p35) +ctrl = CD11c$^+$ cDNA, -ctrl = J774 cDNA; *Il12b* (IL-12/23p40) +ctrl = CD11c$^+$ cDNA, -ctrl = J774 cDNA. Data are represented as mean +/- standard deviation. Not significant (ns): P>0.05, *: 0.01<P<0.05, **: 0.001<P<0.01.

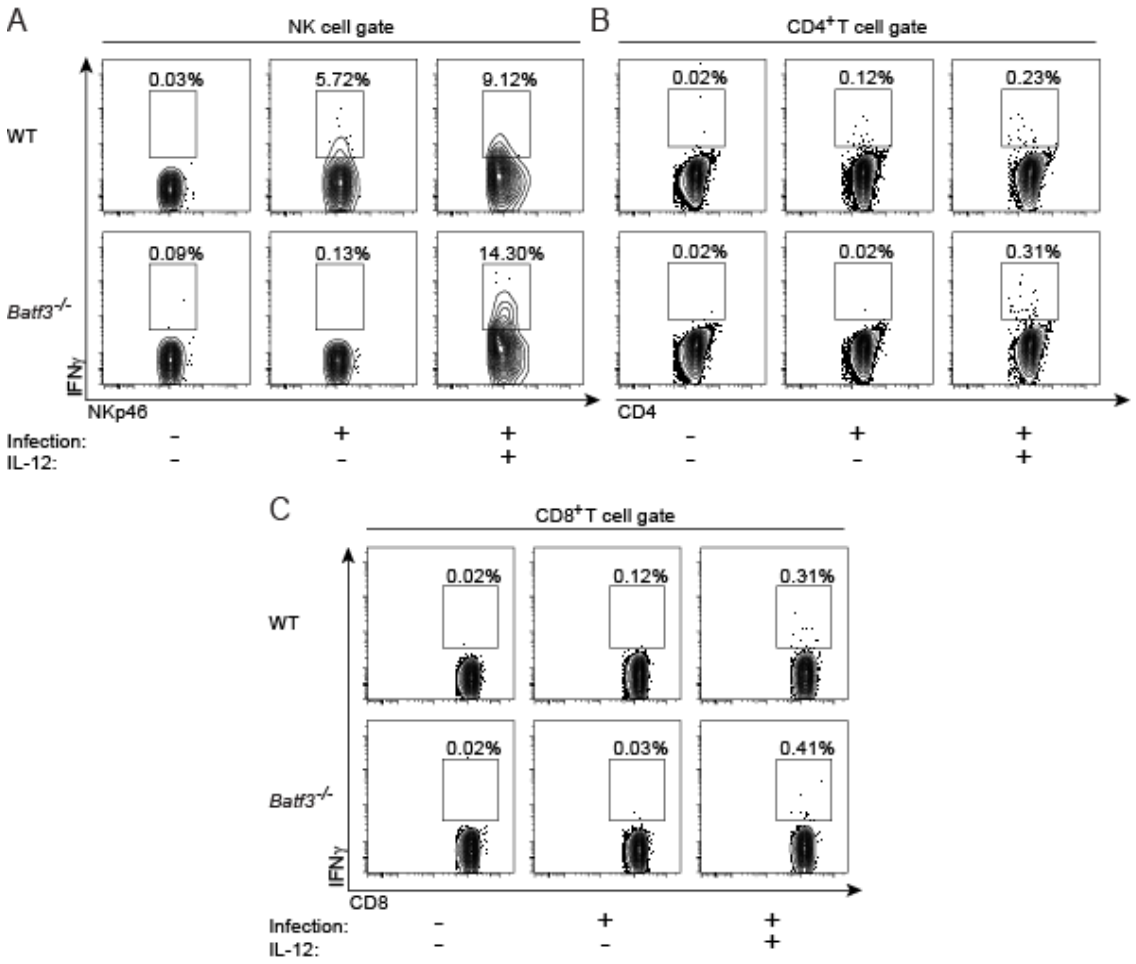

Figure S5, related to Figure 5. Administration of IL-12 restores IFNγ production by NK, CD4$^+$ and CD8$^+$ T cells. Representative flow cytometry plots from Figure 5E-5G. Wild-type and *Batf3*$^{-/-}$ 129S6/SvEV mice were infected with *T. gondii*, given exogenous IL-12 on days 0, 1, 2 after infection, and spleens harvested on days 0 and 3 for analysis by flow cytometry. NK cells gated as NKp46$^+$ Thy1.2$^-$ (A). CD4$^+$ and CD8$^+$ T cells gated as Thy1.2$^+$ and CD4$^+$ (B) or CD8$^+$ (C) (n=3).

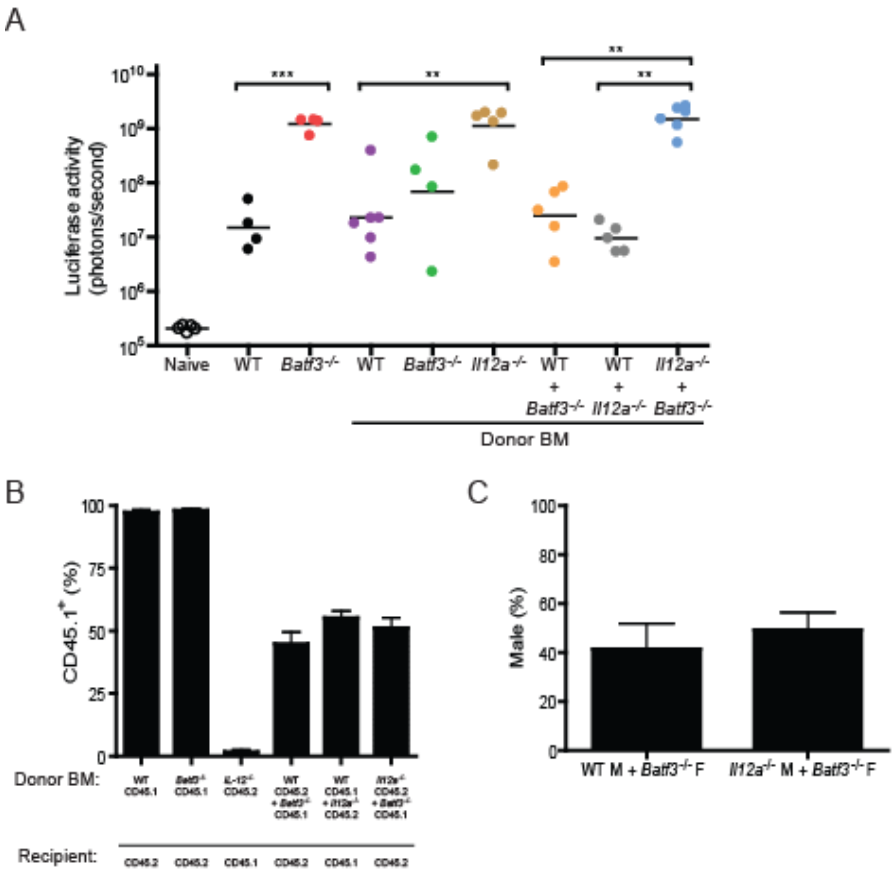

Figure S6, related to Figure 6. Mice re-constituted with a 1:1 mixture of *Batf3*$^{-/-}$ plus *Il12a*$^{-/-}$ bone marrow are unable to control *T. gondii* burden. (A) Parasite burden from repeat of BM chimera experiment in Figure 6 is shown. Non-chimeric C57BL/6 WT and *Batf3*$^{-/-}$ mice were compared with lethally irradiated WT C57BL/6 recipients who were given either WT, *Batf3*$^{-/-}$, or *Il12a*$^{-/-}$ BM, or a 1:1 mixture of WT plus *Batf3*$^{-/-}$, WT plus *Il12a*$^{-/-}$, or *Batf3*$^{-/-}$ plus *Il12a*$^{-/-}$ BM. Whole body *in vivo* bioluminescence imaging was done on day 7 after infection (n=4-6, data representative of 2 independent experiments). Horizontal lines represent the geometric mean. (B and C) Mixed BM chimeras were analyzed after re-constitution to determine chimerism. (B) Blood from C57BL/6 BM chimeras shown in Figure S6 A was analyzed by flow cytometry. The contribution from different donor BM was determined using the congenic markers CD45.1 and CD45.2. (C) Blood from BALB/c BM chimeras shown in Figure 6 was analyzed by quantitative RT-PCR. The contribution from different donor BM was determined using the male Y chromosome gene *Zfy* as a marker. Data are represented as mean +/- standard deviation. **: 0.001<P<0.01, ***: P<0.001.

Supplemental Experimental Procedures:

Oral infection. For oral infection of mice, both the PRU-FLuc-GFP strain and the type II ME49 strain of *T. gondii* expressing a firefly luciferase transgene (ME49-FLuc) (provided by Laura Knoll, University of Wisconsin, Madison, WI) were used. Outbred CD1 mice (Charles River Laboratories, Wilmington, MA) were infected ip with either 1,000 tachyzoites or 5-10 tissue cysts, and used as the source of tissue cysts for experiments 1-3 months after inoculation. To harvest cysts, animals were sacrificed, and the brains were removed and homogenized in 1mL of phosphate buffered saline (PBS). After counting, 5 ME49-FLuc cysts or 20 PRU-FLuc-GFP cysts were administered to experimental animals via oral gavage.

Histology. Mice were sacrificed on day 9 after infection, the spleen and ileum were harvested and fixed in 10% neutral buffered formalin. Tissues were dehydrated in ethanol, embedded in paraffin, and 5μm sections were stained with hematoxylin and eosin (H&E).

Antibodies. The following antibodies were purchased from BD: PE-Cy7 anti-CD11b (M1/70); PE-Cy7 anti-CD4 (RM4-5); APC anti-CD3ε (145-2C11); PerCP-Cy5.5 anti-CD8α (53-6.7); V450 anti-CD8α (53-6.7); APC anti-CD8α (53-6.7); PE-Cy7 anti-CD8α (53-6.7); PE anti-Thy1.2 (30-H12); V450 anti-Ly-6C (AL-21); APC anti-IFNγ (XMG1.2); PE-Cy7 anti-IFNγ (XMG1.2); APC anti-IL-12p40/p70 (C15.6); Rat IgG_1,κ APC (R3-34); V450 anti-Gr1 (RB6-8C5)

The following antibodies were purchased from eBioscience: eFluor 450 anti-MHCII I-A/I-E (M5/114.15.2); APC-eFluor 780 anti-CD11c (N418); APC-eFluor 780 anti-CD4; PE-Cy7

anti-B220 (RA3-6B2); APC-eFluor 780 anti-B220 (RA3-6B2); PE anti-CD103 (2E7); eFluor 450 anti-CD317 (PDCA) (eBio927); eFluor 450 anti-CD335 (NKp46) (29A1.4);

The following antibodies were purchased from Miltenyi: PE anti-DEC205 (NLDC-145); APC anti-DEC205 (NLDC-145); PE anti-Ly-6G (1A8)

Quantitative RT-PCR. For gene expression analysis various cell types were isolated to greater than 99% purity from 129S6/SvEV spleens using a FACSAria II (BD). Total RNA and cDNA were prepared with the RNeasy Micro Kit (Qiagen) and Superscript III reverse transcriptase (Invitrogen). For real-time PCR, StepOnePlus Real-Time PCR System (Applied Biosystems) was used according to the manufacturer's instructions, using the Quantitation, Standard-Curve method and SYBR Green PCR master mix. PCR conditions were 10 min at 95 °C, followed by 40 two-step cycles consisting of 15 s at 95 °C and 1 min at 60 °C.

For analysis of *Tlr11* expression, dendritic cells and neutrophils were identified using the following antibodies: BST2, CD11c, B220, CD4, CD8, GR1 and CD11b. Listed are only the markers for which they express: CD4 DC = $CD11c^+$ $CD4^+$, CD8 DC = $CD11c^+$ $CD8^+$, Plasmacytoid DC = $CD11c^+$ $B220^+$ $BST2^+$, Neutrophils = $GR1^+$ $CD11b^+$. Lymphoid cells were identified using the following antibodies: CD4, CD8, B220, CD11b and CD11c. Listed are only the markers for which they express: CD4 T cells = $CD4^+$, CD8 T cells = $CD8^+$, B cells = $B220^+$. Monocytes were isolated from bone marrow and expressed CD11b and Ly-6C.

For analysis of IL-23p19, IL-12p35 and IL-12/23p40 subunits, $CD8\alpha^+$ DCs were sorted based on the following markers: $BST2^-$ $MHCII^+$ $CD11c^+$ $CD8\alpha^+$ $DEC205^+$. As a control, total splenic $CD11c^+$ cells were purified using Miltenyi microbeads to negatively select for cells expressing B220, Thy1.2, and DX5, followed by positive selection using CD11c (done according

to the manufacturer's protocol). In addition, RNA was harvested from whole kidney lysate as well as from the macrophage cell line J774 to serve as positive and negative controls.

Primers used to evaluate relative expression were as follows: *TLR11* (encoding toll-like receptor 11) TLR11-forward, 5'-TGATGTATTCGTGTCCCACTGC-3', and TLR11-reverse, 5'-CCACTCTTTCTCTCCTCTTCCTCG-3'. *Il23a* (encoding interleukin 23, alpha subunit p19) IL-23p19-forward, 5'- AGCGGGACATATGAATCTACTAAGAGA-3', and IL-23p19-reverse, 5'-GTCCTAGTAGGGAGGTGTGAAGTTG-3'. *Il12a* (encoding interleukin 12, subunit p35) IL-12p35-forward, 5'- TACTAGAGAGACTTCTTCCACAACAAGAG-3', and IL-12p35-reverse, 5'- TCTGGTACATCTTCAAGTCCTCATAGA-3'. *Il12b* (encoding interleukin 12/23, subunit p40) IL-12/23p40-forward, 5'- GACCATCACTGTCAAAGAGTTTCTAGAT-3', and IL-12/23p40-reverse, 5'- AGGAAAGTCTTGTTTTTGAAATTTTTTAA-3' (Uhlig et al., 2006). *Hprt1* (encoding hypoxanthine guanine phosphoribosyl transferase) was used as a normalization control, HPRT-forward, 5'-TCAGTCAACGGGGGACATAAA-3', and HPRT-reverse, 5'-GGGGCTGTACTGCTTAACCAG-3'.

T cell transfer into *Rag2*$^{-/-}$ mice. Wild-type T cells were purified using Miltenyi Thy1.2 microbeads and an LS cell separation column according to the manufacturer's protocol. 10 X 10^6 T cells were adoptively transferred into recipient animals one day prior to infection.

Supplemental References:

Uhlig,H.H., McKenzie,B.S., Hue,S., Thompson,C., Joyce-Shaikh,B., Stepankova,R., Robinson,N., Buonocore,S., Tlaskalova-Hogenova,H., Cua,D.J., and Powrie,F. (2006). Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. Immunity 25, 309-318.