Supporting Information

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SI Materials and Methods

***C. trachomatis* Culture.** *C. trachomatis* serovar L2 (434/Bu; ATCC) or serovar D (UW-3/Cx; ATCC) was propagated within McCoy cells grown in Eagle's MEM (Invitrogen) supplemented with 10% FCS, 1.5 g/L sodium bicarbonate, 0.1 mM nonessential amino acids, and 1 mM sodium pyruvate. Cells were lysed with sterile glass beads and sonicated to release bacteria from the inclusion. Elementary bodies (EBs) were purified by density gradient centrifugation as previously described (7, 8), stored at −80 °C, then thawed immediately before use. Inclusion forming units (IFUs) of thawed EBs were titered on McCoy cell monolayers.

Mice. C57BL/6J and B6.129S4-*Cxcl10*^{tm1Adl}/J mice were purchased from The Jackson Laboratory and housed at the Harvard Medical School Center for Animal Resources and Comparative Medicine. All mouse procedures were performed in accordance with protocols approved by Harvard's Institutional Animal Care and Use Committee. Transcervical infections were performed as has been previously described (7, 8). Briefly, 6- to 8-wk-old female mice were treated s.c. with 2.5 mg medroxyprogesterone acetate (Pfizer) 1 wk before infection with 5×10^6 IFU of purified *C. trachomatis* serovar L2 (434/Bu) or serovar D (UW-3/Cx) EBs. Bacteria were introduced transcervically (t.c.) into the upper genital tract using a nonsurgical embryo transfer device (ParaTechs). At time points described in the text, the upper genital tract was harvested and processed for flow cytometry or submitted to the Rodent Histopathology Core Facility at Harvard Medical School. In accordance with best practices in the histopathology literature (9), harvested tissue was assigned a severity score based on the prevalence of pathology phenotypes relevant to human female upper genital tract infection with *C. trachomatis* (4), including fibrosis, edema, epithelial/membrane thickening and/or degeneration, luminal/tissue cellular infiltration, and increased vascularity. Scores range from 0 (no pathology); 1 (mild/rare pathology, less than 1/3 of tissue affected); 2 (moderate/multifocal pathology, between 1/3–2/3 of tissue affected); to 3 (severe/coalescing pathology, greater than 2/3 of tissue affected).

Quantitative PCR. The bacterial burden of Ct was determined using a quantitative PCR assay that has been shown previously to accurately reflect levels of Ct inclusion forming units (see figure 2 in ref. 7). DNA from upper genital tract homogenates was isolated using a DNeasy Blood and Tissue Kit (Qiagen). Ct 16S DNA and mouse GAPDH DNA was amplified and quantitated on an ABI7000 (Applied Biosystems) using specific primer pairs and probes (IDT or Applied Biosystems). The ratio of Ct/mouse DNA was calculated using standard curves generated from known amounts of purified Ct or mouse DNA.

Flow Cytometry. Murine upper genital tracts were processed into single-cell suspensions as previously described (6). Cells were then preincubated with CD16/CD32 (2.4G2; Bio X-Cell) before staining with fluorochrome-conjugated antibodies against mouse CD4 (RM4-5 or GK1.5), CD8 (53-6.7), CD90.1 (OX-7), CD90.2 (53-2.1), CD3 (17A2), CD11b (M1/70), CD11c (HL3 or N418), NK1.1 (PK136), Gr1 (RB6-8C5), Ly6G (IA8), Ly6C (AL-21), B220 (RA3-6B2), F480 (BM8), or MHCII (AF6-120.1). All antibodies were purchased from BioLegend or Invitrogen. Live/dead stain kit (Invitrogen) was used to exclude dead cells and AccuCheck Counting Beads (Invitrogen) were used to quantify the absolute cell number in each sample. Data were collected on a LSRII (BD Bioscience) and analyzed using FlowJo (Tree Star Industries).

Detection of *C. trachomatis*-Specific T Cells. Ct-specific CD8$^+$ T cells were detected with D^b/ASFVNPIYL (CrpA$_{63–71}$) MHC class I tetramer (National Institutes of Health Tetramer Facility). The MHC class II tetramer used previously to detect Ct-specific CD4$^+$ T cells in lymphoid tissue (7) is not sensitive enough to detect these cells in the uterus so transgenic CD90.1$^+$ Ct-specific CD4$^+$ T cells (7) were transferred i.v. (5×10^5 per mouse) into naïve CD90.2$^+$ C57BL/6 recipient mice 1 d before infection. The presence of these cells in the upper genital tract was detected using anti-CD90.1 antibody.

Measurement of Gene Expression by RT-qPCR. RNA was extracted from upper genital tract homogenates with the RNeasy kit (Qiagen) and used as template for RT-PCR. Expression of the following genes was detected using the QuantiTect SYBR Green RT-PCR kit (Qiagen) with verified primers from PrimerBank (https://pga.mgh.harvard.edu/primerbank/): CXCL9 (F-5′ GGAGTTCGAGGAACCCTAGTG; R-5′ GGGATTTGTAGTGGATCGTGC); CXCL10 (F-5′ CCAAGTGCTGCCGTCATTTTC; R-5′ GGCTCGCAGGGATGATTTCAA); and CXCL11 (F-5′ GGCTTCCTTATGTTCAAACAGGG; R-5′ GCCGTTACTCGGGTAAATTACA). GAPDH message was quantified for normalization using primers F-5′ GGTGCTGAGTATGTCGTGGA; R-5′ CGGAGATGATGACCCTTTTG.

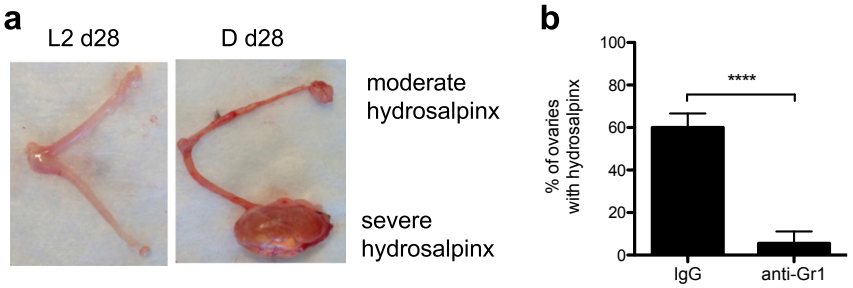

Fig. S1. *C. trachomatis* serovar D-infected mice present with gross hydrosalpinx, which can be ameliorated by neutrophil depletion. (*A*) Gross pathology of mouse upper genital tracts at day 28 postinfection with Ct L2 (*Left*) or Ct D (*Right*), showing moderate-to-severe hydrosalpinx only in Ct D-infected upper genital tracts. (*B*) Assessment of the presence or absence of gross hydrosalpinx in mice infected with Ct D and treated with isotype control IgG or anti-Gr1 neutrophil-depleting antibody. Data in *B* shown as the mean ± SEM of at least 10 mice per group per treatment. ****$P < 0.0001$ (Student's *t* test).

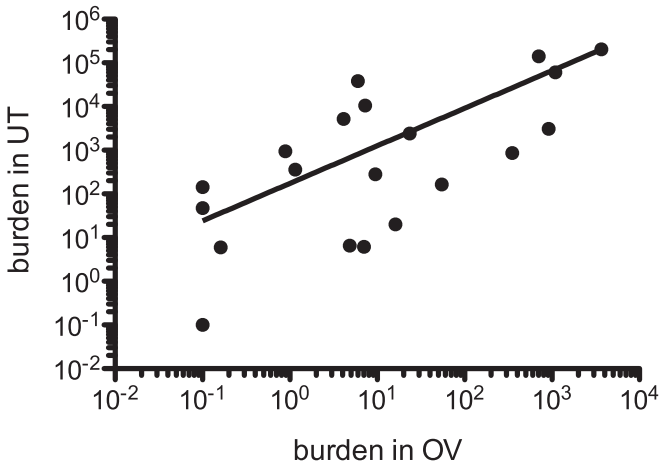

Fig. S2. Significant correlation between *C. trachomatis* burden in the uterus vs. ovaries. The upper genital tracts of *C. trachomatis*-infected mice were bisected at the oviduct, which subdivided the tissue into the uterus or two ovaries. The bacterial burden was quantified by qPCR separately for each tissue type, plotted for each mouse, and found to significantly correlate between uterine vs. ovarian tissues (Spearman's correlation, $P = 0.0032$). Data are combined from two independent experiments at days 3 and 6 postinfection.

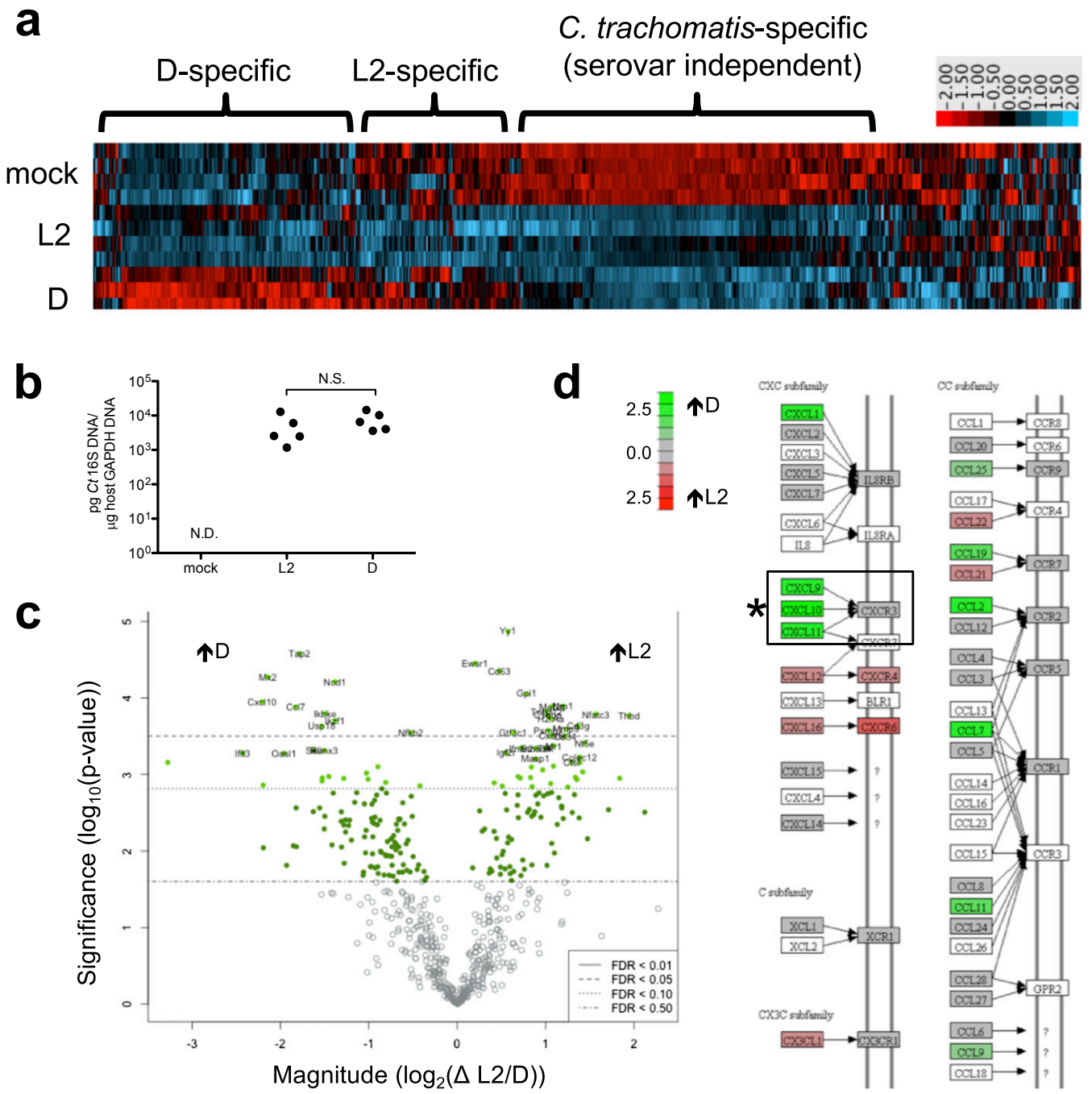

Fig. S3. Uterine gene expression after infection with *C. trachomatis* reveals serovar-specific responses, including a node of interaction between CXCL9/10/11 and their common receptor, CXCR3. RNA was isolated from the upper genital tract 2 d after Ct L2, Ct D, or mock infection and applied to the NanoString PanCancer Immune Profiling Panel. (*A*) Heat map of *z* scores color-coded by logtwofold change (key), where each row represents a mouse (infection condition noted on the *Left*); each column represents one of 700+ genes. The intensity of blue/red color denotes the degree to which an individual's expression level differs from the mean expression level (colored black) as measured in SDs. Genes are grouped according to similarity in *z* score by the algorithm and clearly cluster in agreement with infection condition (mock, Ct L2, or Ct D). (*B*) Bacterial burden in the upper genital tract. N.S., not significant. (*C*) Volcano plot showing direct comparison of gene expression after Ct D vs. Ct L2 infection. Each dot represents one gene; FDR, false discovery rate. (*D*) Serovar-specific changes in gene expression color-coded by *z* score and plotted onto the KEGG chemokine and cytokine pathway. *, up-regulation of CXCL9-11 after Ct D infection.

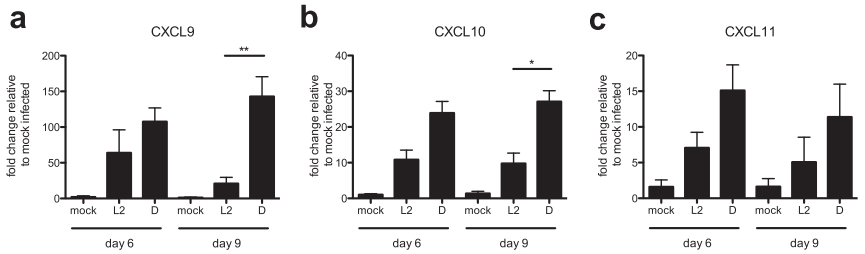

Fig. S4. The expression of CXCL9/10/11 remains elevated at later time points in the upper genital tracts of *C. trachomatis* D- vs. L2-infected mice. RNA was extracted from the upper genital tracts of mock-infected, Ct L2-infected, or Ct D-infected mice at days 6 and day 9 postinfection. The expression of CXCL9 (*A*), CXCL10 (*B*), and CXCL11 (*C*) was quantified by RT-PCR, normalized to GAPDH levels, and then calculated as fold change over mock-infected mice. Data are shown as the mean ± SEM of three mice per mock-infected group and five mice per Ct-infected group; $*P < 0.05$, $**P < 0.01$ (Student's *t* test).

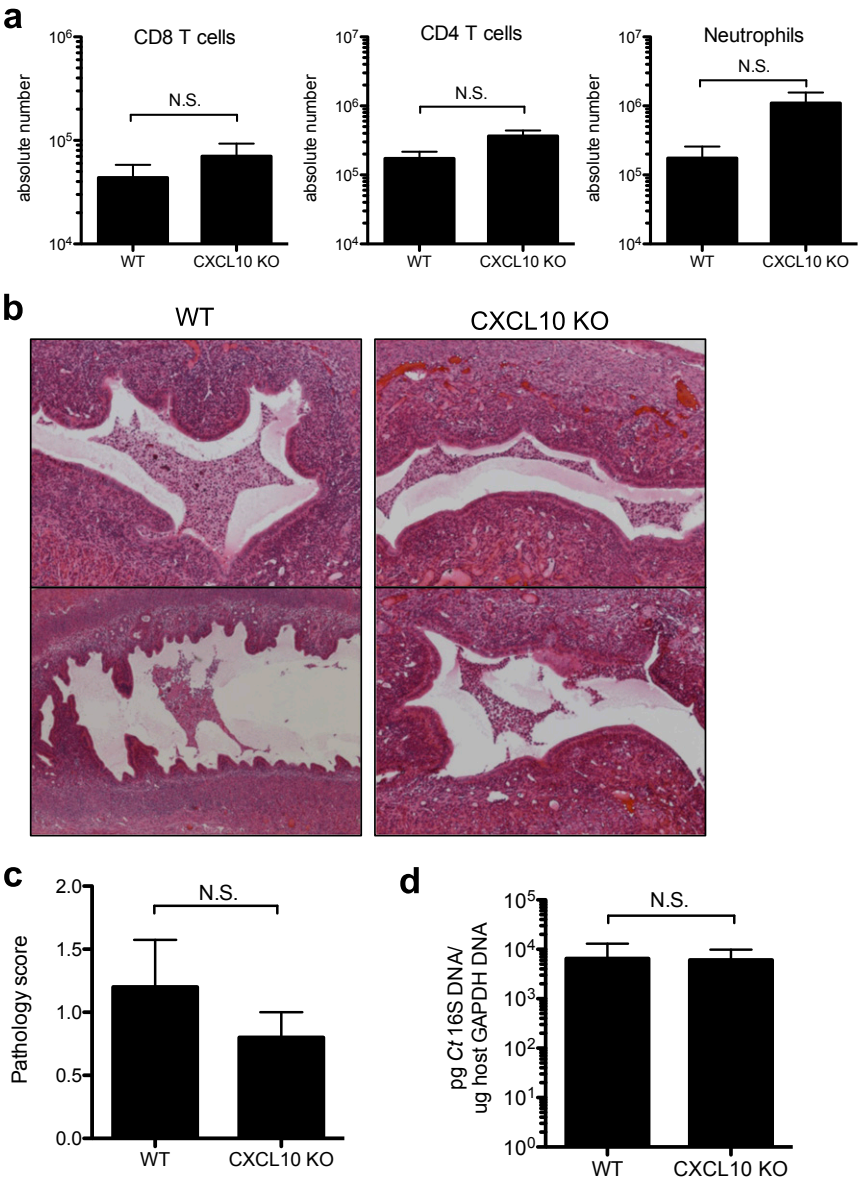

Fig. S5. Deletion of CXCL10 is not sufficient to affect cellular infiltration or tissue pathology following infection with *C. trachomatis*. Ct D was used to infect wild-type (WT) mice or CXCL10$^{-/-}$ mice. (*A*) Quantification by flow cytometry of the amount of each of the indicated cell types in the upper genital tract at day 9 postinfection. (*B*) Representative H&E-stained sections of Ct D-infected uteri from WT or CXCL10$^{-/-}$ mice. (*C*) Upper genital tract immunopathology on a 0–3 scale (0, none; 1, mild; 2, moderate; and 3, severe), showing no significant difference in pathology. (*D*) Bacterial burden in the upper genital tract, showing no effect of CXCL10 deletion on *C. trachomatis* load. Data in *A*, *C*, and *D* are shown as the mean ± SEM of at least five mice per group per treatment; N.S., not significant (Student's *t* test).

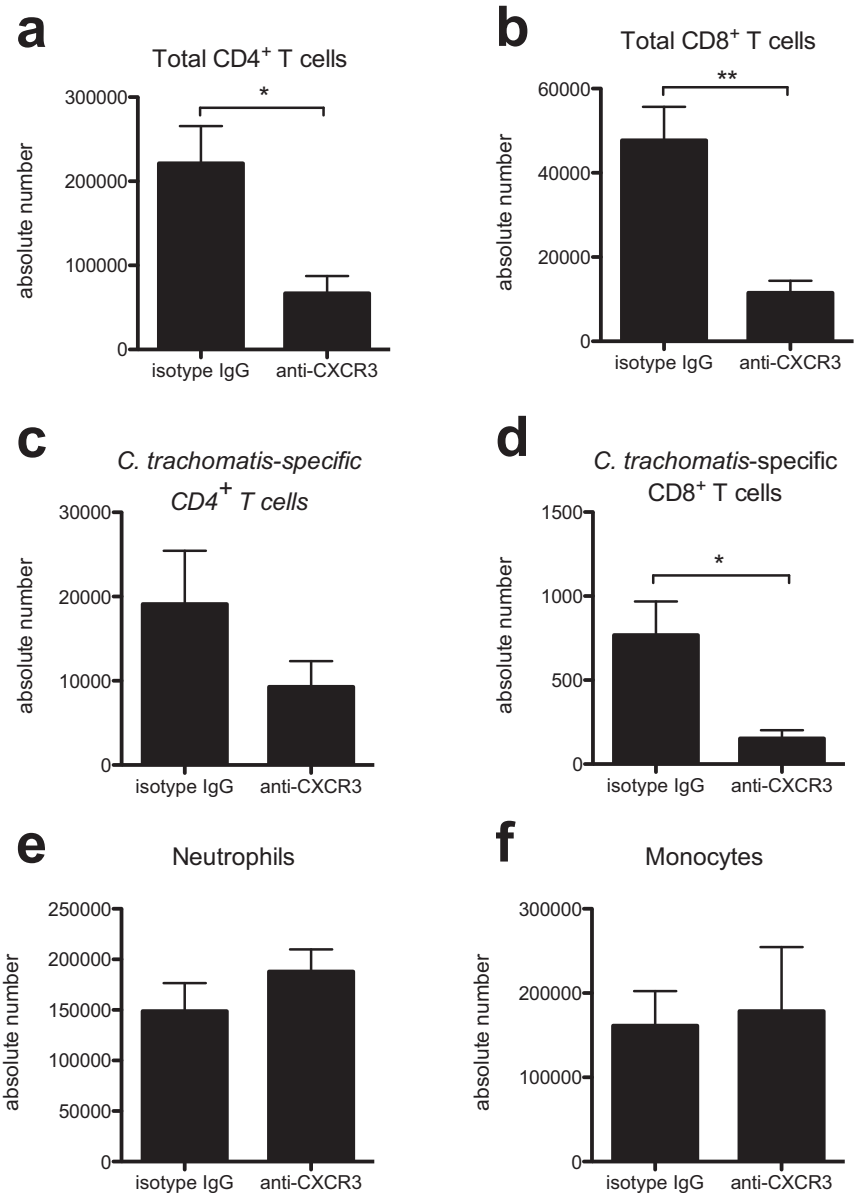

Fig. S6. CXCR3 blockade more significantly inhibits the recruitment of total $CD4^+$ and $CD8^+$ T cells than *C. trachomatis*-specific $CD4^+$ and $CD8^+$ T cells to the *C. trachomatis*-infected upper genital tract. Ct D-infected mice were treated with anti-CXCR3 monoclonal antibody or matching isotype control (IgG). The number of total $CD4^+$ T cells (*A*), total $CD8^+$ T cells (*B*), Ct-specific $CD4^+$ T cells (*C*), Ct-specific $CD8^+$ T cells (*D*), neutrophils (*E*), and monocytes (*F*) in the upper genital tract was quantified by flow cytometry 7 d after infection. Data are shown as the mean ± SEM of five mice per group; $*P < 0.05$, $**P < 0.01$ (Student's *t* test).

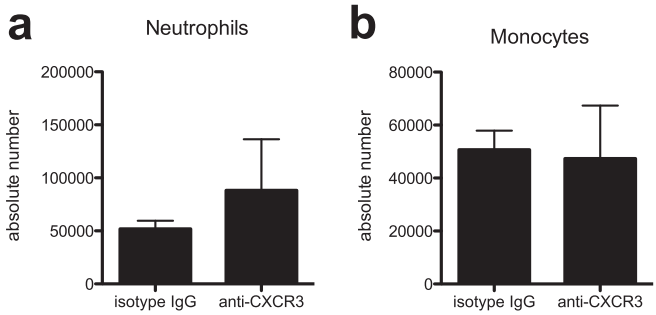

Fig. S7. Antibody blockade of CXCR3 has no effect on neutrophil recruitment to the upper genital tract. Ct D-infected mice were treated with anti-CXCR3 monoclonal antibody or matching isotype control (IgG). The number of neutrophils (*A*) and monocytes (*B*) in the upper genital tract were quantified by flow cytometry 5 d after infection. Data are shown as the mean ± SEM of five mice per group; no significant differences were observed (Student's *t* test).

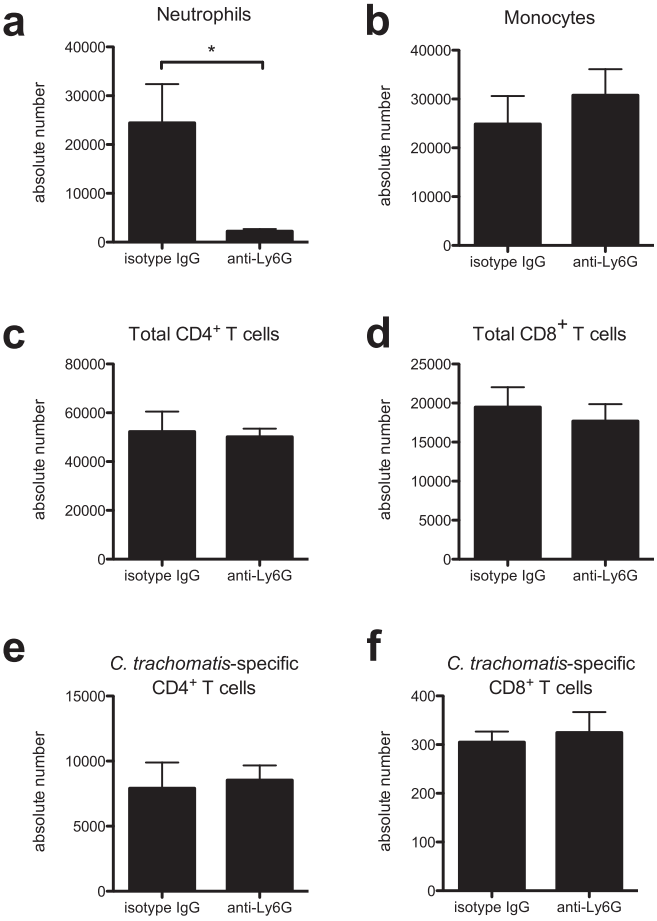

Fig. S8. Depletion of neutrophils has no effect on the number of total or *C. trachomatis*-specific $CD4^+$ or $CD8^+$ T cells in the upper genital tract. Ct D-infected mice were treated with anti-Ly6G monoclonal antibody or matching isotype control (IgG). The number of neutrophils (*A*), monocytes (*B*), total $CD4^+$ T cells (*C*), total $CD8^+$ T cells (*D*), Ct-specific $CD4^+$ T cells (*E*), and Ct-specific $CD8^+$ T cells (*F*) in the upper genital tract was quantified by flow cytometry at day 6 post-infection. Data are shown as the mean ± SEM of five mice per group; $*P < 0.05$ (Student's *t* test).

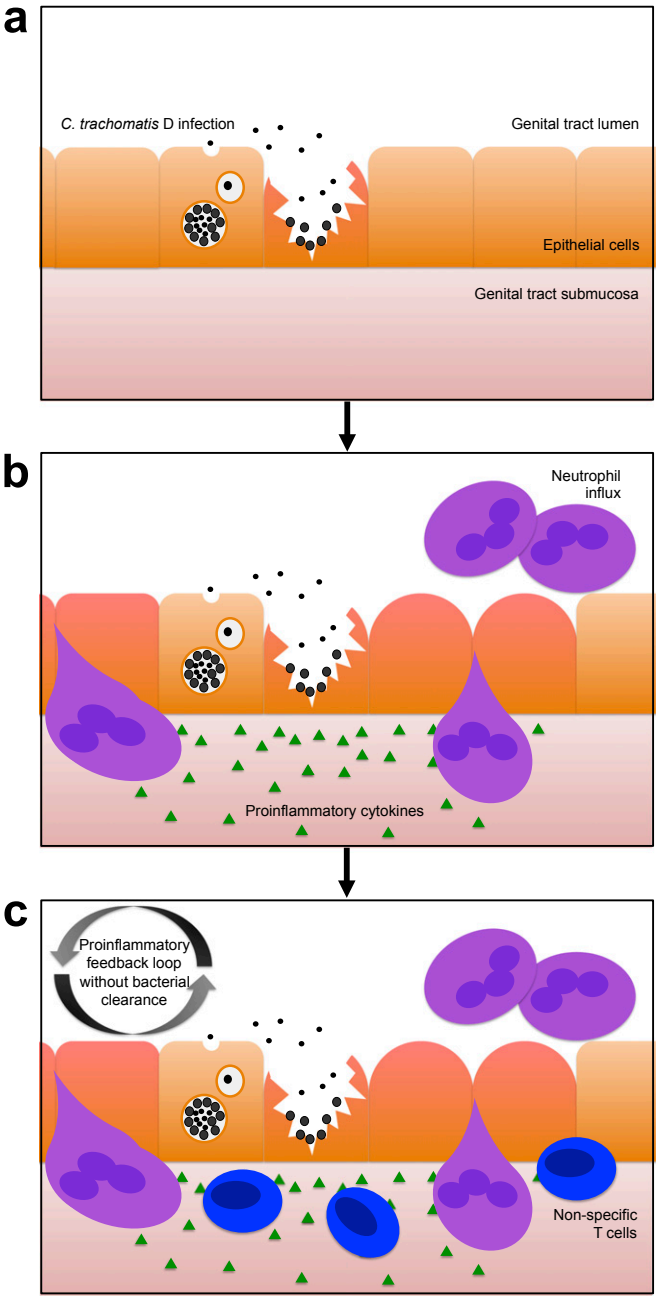

Fig. S9. Working model of *C. trachomatis*-induced immunopathology in the upper genital tract. (*A*) *C. trachomatis* infection of the genital mucosa: use of genital-specific serovar D (small black circles) in the mouse upper genital tract results in immunopathology (reddened, rounded epithelial cells) reminiscent of human female genital tract disease sequelae. (*B*) Early-stage immunopathology (days 2–6 postinfection): *C. trachomatis*-induced genital tract inflammation is characterized by proinflammatory chemokine expression (small green triangles) and massive infiltration of neutrophils (purple polymorphonuclear cells) to the tissue, required for pathology but not protection. (*C*) Late-stage immunopathology (days 6–9+): Chemokine-dependent recruitment of nonprotective T cells (blue mononuclear cells) to the genital mucosa perpetuates immunopathology without contributing to bacterial clearance.