## Supporting Information

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**SI Materials and Methods**

***Tissue preparation.*** *Embryonic tissue.* Pregnant female mice were sacrificed by cervical dislocation. Embryos (E12.5–E18.5) were harvested from the uterus and washed with cold phosphate buffered saline (PBS; PAA Laboratories, Cölbe, Germany). Epididymides and testes were carefully dissected from the decapitated male embryos. Single cell suspensions were obtained by enzymatically digesting epididymides and testes by shaking for 30 min at 37˚C in RPMI 1640 medium containing collagenase I (1 mg/ml; Roche, Munich, Germany), DNase I (1 unit/ml, Roche) and 3% fetal calf serum (PAA Laboratories). The digested cells were filtered through a 70 µM cell strainer and pelleted by centrifugation at 350 g for 7 min, 4°C.

***Adult tissue****.* Pre-pubertal mice (postnatal day (PND) 3 – PND 14) were sacrificed by cervical dislocation, whereas post-pubertal and adult mice were euthanized by cervical dislocation in deep isoflurane anesthesia. Blood was collected from the vena cava, and erythrocytes were immediately lysed with RBC lysis buffer (Qiagen, Hilden, Germany). Epididymides, testes, intestine, liver, and brain were harvested for flow cytometric analysis. The testicular capsule was removed and the intestine was cleared of feces. To obtain single cell suspensions, harvested organs were finely minced and enzymatically digested for 30 min at 37˚C in RPMI 1640 medium containing collagenase I (1 mg/ml), DNase I and 10% fetal calf serum. The digested cells were filtered through a 70 µM cell strainer and pelleted by centrifugation at 350 g for 7 min at 4°C.

**Antibodies and flow cytometric analysis**. Single cell suspensions were incubated with a Fc blocker (anti-CD32/16; BD Bioscience, San Jose, CA, USA) antibody for 30 min, and then incubated with the respective mix of antibodies (Table S1) for 30 min at 4˚C. After

labeling, cells were washed with PBS and resuspended in FACS buffer (2 mM EDTA, 2% FCS in PBS) before analysis. Macrophage populations were always gated on single and live cells, with dead cells excluded by DAPI staining. Flow cytometry analysis was performed using a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany) or a BD Biosciences LSR Fortessa (BD Bioscience). Data were analyzed with the FlowJo software version X (Tree Star, Ashland, OR, USA).

**Parabiosis:** Parabiosis between CD45.1$^+$ and *Ccr2*$^{-/-}$CD45.2$^+$ male mice were established as described previously (1). After surgery, mice were injected sub cutaneously (*s.c*.) with 0.1mg/kg of buprenorphine *f*ollowed by another injection 24hr later. Mice were maintained on *s.c*. saline injection for 1 week with additional 3% neomyocin antibiotics for 2 weeks. Mice were sacrificed after 6 months of parabiosis to analyze chimerism in different organs. To analyze the contribution of blood monocyte in inflammatory condition, 8 to 9 weeks old, weight matched wild type CD45.1$^+$ and CD45.2$^+$ male mice were sutured together for 8 weeks. After surgery, mice received sulfamethoxazole (2 mg/mL) and trimethoprim (0.4 mg/mL) antibiotics for 1 week in normal drinking water. After 8 weeks, mice were injected with LPS (0.2 mg/kg *(*b.w.*) and* sacrificed 18 h later to determine the chimerism in the epididymis and testis.

**Models of testicular and epididymal inflammation.** *UPEC induced epididymo-orchitis model.* Epididymo-orchitis was induced by infection with a uropathogenic *Escherichia coli* (UPEC strain CFT073) as previously described(2). Adult male mice were anesthetized by ketamine (100 mg/kg b.w., Bela-Pharm, Vechta, Germany) and xylazine (10 mg/kg b.w.) and UPEC or saline was injected into the lumen of each vas deferens, close to the epididymides. Before the injection, vasa deferentia were ligated at the site of injection to

prevent spreading of infection to the accessory sex glands and urethra. Seven days post infection, organs were retrieved for analysis by flow cytometry and immunohistochemistry.

**LPS administration**. Lipopolysaccharide (LPS) (0.2 mg/kg b.w., Sigma) was diluted in PBS and administered i.p. Mice were euthanized after 18 h to analyze the infiltration of immune cells into the epididymis and testis.

**Histological analysis.** UPEC infected testes and epididymides were fixed in Bouin's fixative for 4 h and then embedded in paraffin. Tissue sections (5 µm) were stained with Sirius Red (epididymis) or hematoxylin and eosin (testis). After staining, images were acquired with a Leica DM750 microscope (Leica Microsystems, Wetzlar, Germany).

**BrdU incorporation assay.** To determine the proliferation of macrophages after UPEC infection mice were kept on drinking water containing BrdU (0.8 mg/ml, Sigma) throughout the course of the experiment. To determine the proliferative capacity of macrophages *in situ* in normal and LPS challenged testes, mice were injected i.p. with BrdU (0.1 mg/kg body weight) 4 h before euthanasia and organ retrieval. BrdU staining was analyzed in macrophage populations by flow cytometry as described above.

**Macrophage isolation.** To sort macrophage populations, 9-10 week old C57BL/6J male mice were terminally anesthetized by ketamine and xylazine. Mouse hearts were perfused with 10 ml of cold PBS and then brains, epididymides and testes were harvested. Cell suspensions from harvested organs were obtained as described above. Single cell suspensions from 7 testes and epididymides were pooled in separate tubes. Cells from brains, epididymides, and testes were filtered through a 70 µm cell strainer and resuspended into 40% isotonic Percoll (GE Healthcare, Uppsala, Sweden) at a density of

1.072 g/ml in PBS containing 3% FCS. Cells were collected from 1.06 and 1.072 g/ml layers after centrifugation and resuspended. Erythrocytes were lysed in RBC lysis buffer (Qiagen). The cells were blocked with FC-block (anti-mouse CD16/32 Ab, 0.1 ug/ml) and labeled with antibodies against CD45, Ly6c, CD11b, CD11c, CD11b and F4/80. Macrophage populations were identified and sorted as live ($DAPI^-$), $CD45^+$ $Ly6c^-$ $CD11c^-$ $CD11b^+$ $F4/80^+$ cells using a BD FACS Aria III (BD Bioscience) cell sorter. The sorted cells were collected in RPMI medium containing 10% FCS.

**Library preparation.** RNA was isolated using the RNeasy Micro Kit (Qiagen) and sequenced using the QuantSeq 3' mRNA-Seq Library Prep Kit (Lexogen, Vienna, Austria) followed by a single end 75-base sequencing run for 75 cycles on a SR HiSeq Rapid V2 flowcell on aq HiSeq1500 (Illumina).

**Bioinformatic analysis**: The sequenced samples were pseudo aligned against the murine reference transcriptome version GSE63341 using Kallisto (3, 4), Kallisto counts were directly imported and normalized using DEseq2 (5). Normalized counts were rlog transformed and the Group Fold Change (GFC) was calculated, which is the mean of each condition compared to the overall mean for each gene respectively. The GFC was used to define tissue specific differences by ordering the respective GFC and selecting the top 20 differentially expressed genes for each tissue. These tables were also filtered by know transcriptional regulators (TR) to get tissue specific TRs. The macrophage data set is available under GSE132471.

**Generation of *Myb* bone marrow chimera.** *Myb* bone marrow chimeras were established as described (6). For deletion of *c-myb* floxed allele, *CD45.2; $Myb^{f/f;}$ $Mx1^{Cre}$* mice carrying a myxovirus resistance 1-Cre cassette (Mx1-Cre) were injected five times

i.p. with polyinosinic-polycytidylic acid (poly(I:C); 10 µg/g b.w.;Sigma) every other day for 10 days. In order to achieve donor *Myb* chimerism, CD45.2; $Myb^{f/f}$; $Mx1^{Cre}$ mice were injected intravenously with $1x10^7$ *Cd45.1; Myb*$^{+/+}$ BM cells (6). Brains, epididymides, testes, and blood were analyzed 9 months after transplantation by flow cytometry as described above.

**Statistical Analyses.** All statistical analyses were performed with GraphPad Prism Software. The statistical analysis applied in each case described in the respective figure legend.

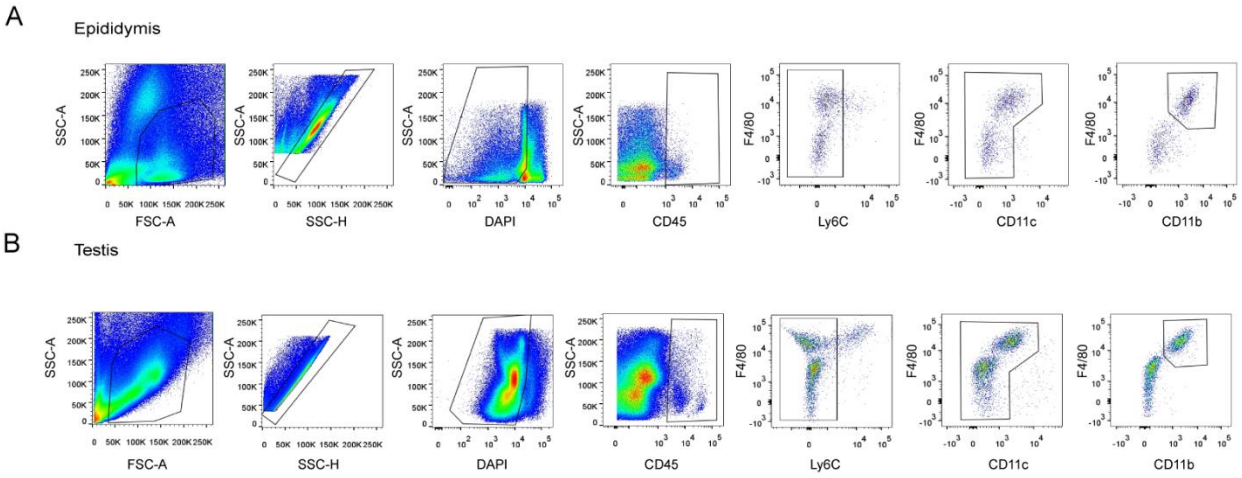
**Reference**.


1. Dick SA*, et al.* (2019) Self-renewing resident cardiac macrophages limit adverse remodeling following myocardial infarction. *Nat Immunol* 20(1):29-39.
2. Klein B*, et al.* (2020) Differential tissue-specific damage caused by bacterial epididymo-orchitis in the mouse. *Mol Hum Reprod* 26(4):215-227.
3. Bray NL, Pimentel H, Melsted P, & Pachter L (2016) Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol* 34(5):525-527.
4. Lavin Y*, et al.* (2014) Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell* 159(6):1312-1326.
5. Love MI, Huber W, & Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12):550.
6. Stremmel C*, et al.* (2018) Inducible disruption of the c-myb gene allows allogeneic bone marrow transplantation without irradiation. *J Immunol Methods* 457:66-72.

**Legend for dataset S1**

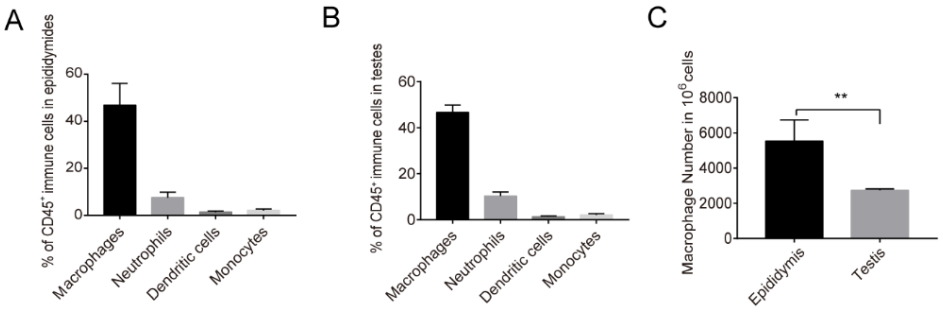
List of differentially-regulated genes in macrophage populations of epididymis, testis and brain.

**Figure S1**



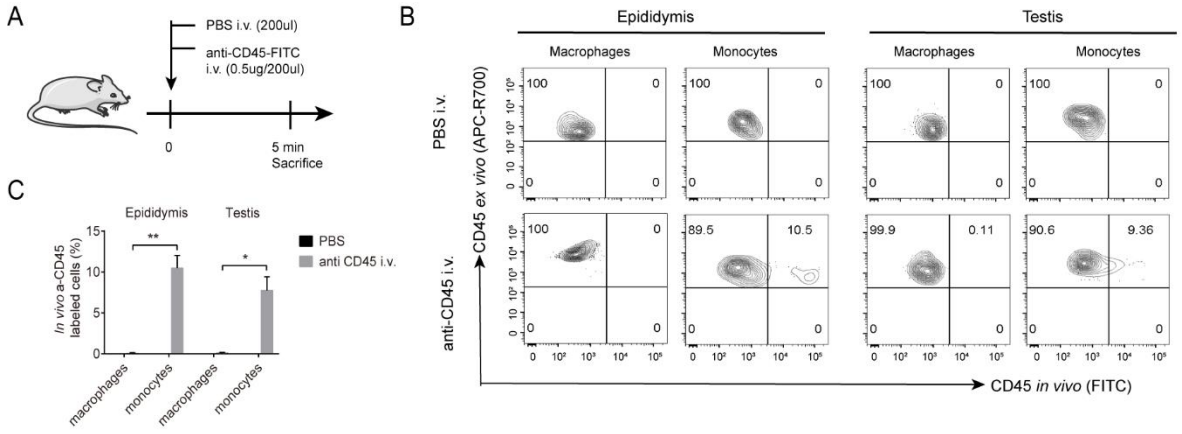

Single cell suspensions of **(A)** postnatal epididymis and (**B**) testis were analyzed by flow cytometry. After excluding cell aggregates and dead cells on the basis of SSC-A and DAPI staining, respectively, whole leucocyte populations were selected by their expression of CD45. Monocytes were excluded by gating out Ly6c$^{+}$ cells, and dendritic cells were gated out by excluding CD11c$^{+}$F4/80$^{-}$ cells. After exclusion of monocytes and dendritic cells, expression of F4/80 and CD11b was determined in cells from epididymis **(A)** and testis **(B)**.

**Figure S2**



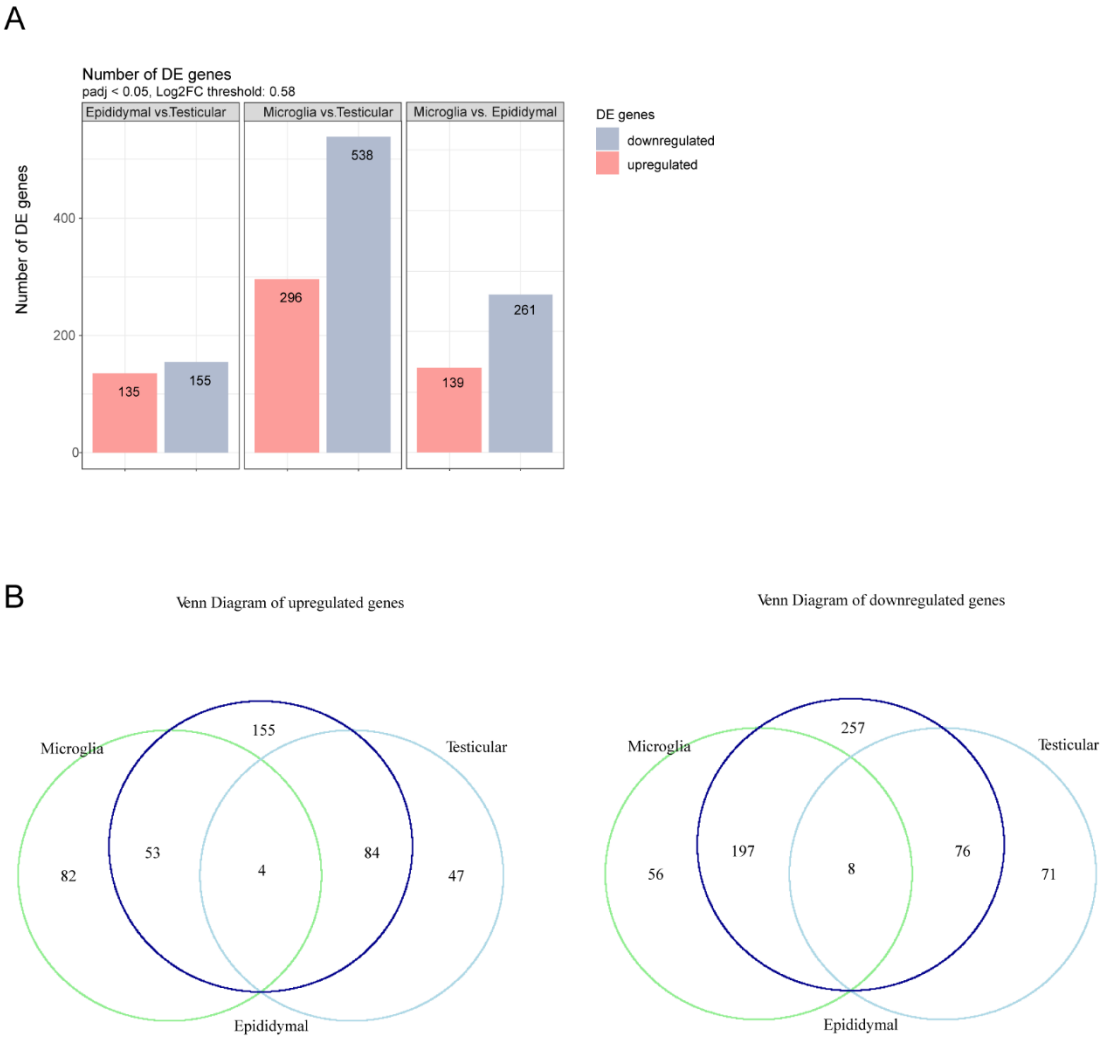

Percentage of macrophages, neutrophils, dendritic cells and monocytes in live CD45$^+$ immune cells was measured in cell suspension from **(A)** epididymis and **(B)** testis of nine week old male mice. Data were obtained from 3-5 individual mice. **(C)** Absolute number of macrophages in epididymis and testis were compared (n=3-5). The Welch's t-test was employed for statistical analysis. Mean ± SD ** $p < 0.01$.

**Figure S3**



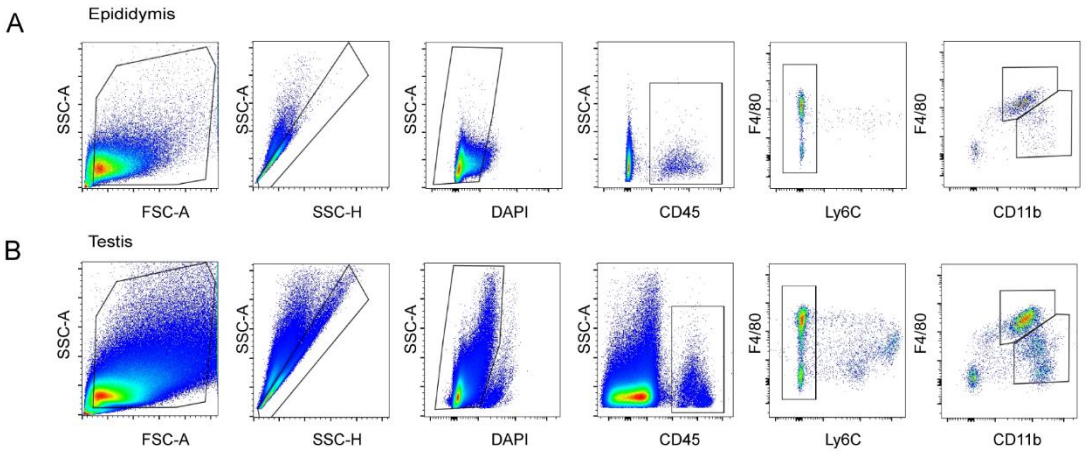

(A) Intravascular leucocytes were labeled by injecting (i.v.) FITC-conjugated anti-CD45 antibodies 5 min before sacrificing the mice (n=3). (B) Representative flow cytometry contour plots show labeling of tissue macrophages (CD45-APC) and intravascular monocytes (CD45-FITC). (C) Percentage of CD45-FITC labeling in macrophage and monocyte populations in epididymis and testis (n=3; Welch's t-test), Mean ± SD, * $p < 0.05$ ** $p < 0.01$.

**Figure S4**



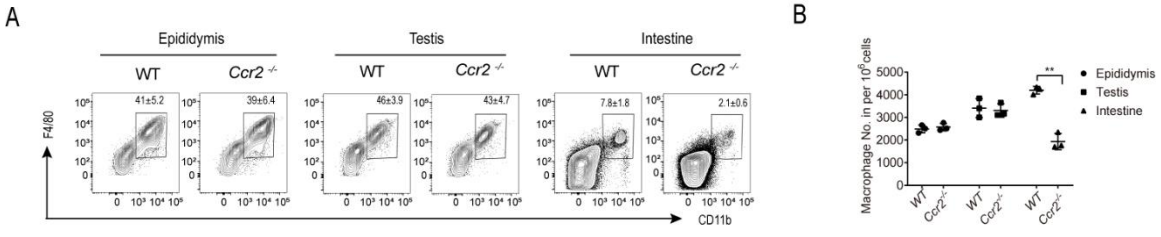

(**A**) The number of differentially-expressed genes (DEG) in macrophage populations of epididymis, testis, and brain; (**B**) Venn diagram of significantly DEG in macrophage populations of epididymis, testis, and brain.

## Figure S5



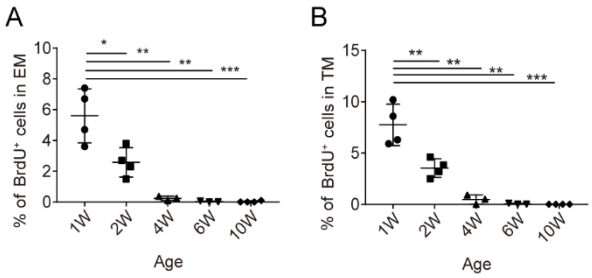

Single cell suspensions of **(A, B)** embryonic epididymis and testis were analyzed by flow cytometry. After excluding cell aggregates and dead cells on the basis of SSC-A and DAPI staining, respectively, whole leucocyte populations were selected by their expression of CD45. After exclusion of Ly6c$^{+}$ monocytes, CD45$^{+}$ cells were further stratified to examine the expression of F4/80 and CD11b in the (**A**) epididymis and (**B)** testis**.**

## Figure S6



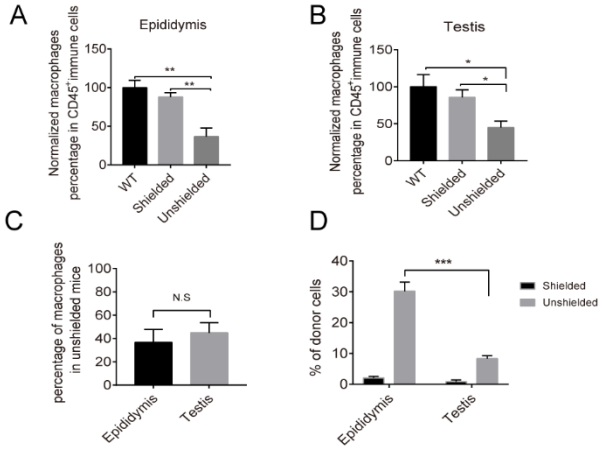

**(A)** Frequency of the F4/80$^{+}$CD11b$^{+}$ macrophage populations within CD45$^{+}$ live cells of epididymis, testis and intestine in 9 week old WT and *Ccr2*$^{-/-}$ mice. **(B)** Frequency of the epididymal, testicular, and intestinal macrophage populations in wild type (WT) and *Ccr2*$^{-/-}$ *mice*. The Welch's t-test was employed for statistical analysis. Mean ± SD, ** $p < 0.01$ (n=3).

**Figure S7**



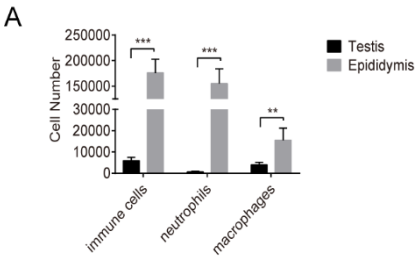

The proliferation rates of **(A)** epididymal (EM) and **(B)** testicular macrophages (TM) were analyzed by flow cytometry 4 hours after administration of BrdU *(i.p.)* in C57BL/6 male mice at the indicated age of animals. Data were obtained from 3-4 individual mice. The Welch's t-test was employed for statistical analysis. Mean ± SD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

**Figure S8**



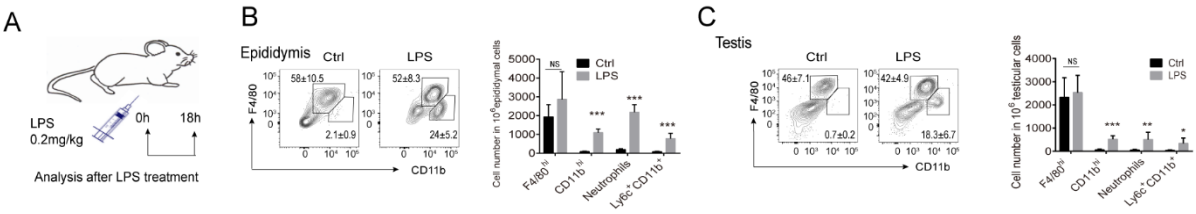

(**A-C**) Six week old C57BL/6J mice were lethally irradiated with or without the inguinal/scrotal region shielded, and one day later the degree of macrophage ablation was determined in (**A**) epididymis and (**B**) testis and (**C**) compared with each other. (**D**) Six week old C57BL/6J mice were lethally irradiated with or without the inguinal/scrotal region shielded, before $EGFP^+$ bone marrow cells were injected into the tail vein. After 12 weeks the frequency of $EGFP^+$ cells was compared in epididymis and testis (one way ANOVA; mean ± SD; n=5, *** $p < 0.0001$, N.S: not significant).

**Figure S9**



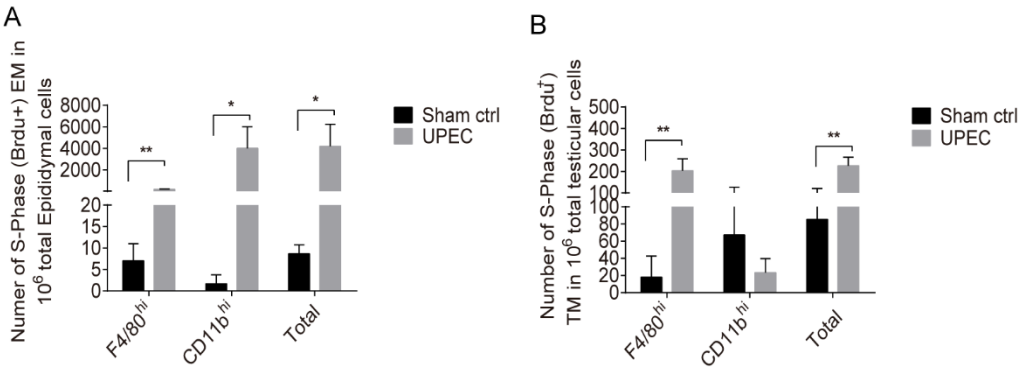

Wild type C57BL/6J mice were treated with saline or UPEC. Seven days post-infection absolute numbers of macrophages, neutrophils, and monocytes were determined in live $CD45^+$ cell from epididymis and testis. Pooled data from 4-6 mice are shown (Welch's t-test; mean ± SD, ** $p < 0.001$, *** $p < 0.0001$).

**Figure S10**




**(A)** WT C57BL/6J mice were injected with PBS or LPS and after 18 h macrophage populations from **(B)** epididymis and **(C)** testis were measured by flow cytometry. Mean numbers of $F4/80^{hi}CD11b^{lo}$ resident macrophages $F4/80^{lo}CD11b^{hi}$ monocyte-derived macrophages, $Ly6G^+$ neutrophils, and $Ly6C^+CD11b^+$monocytes are presented (Welch's t-test, mean ± SD, n=5 for each group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$).

**Figure S11**




Number of macrophages from **(A)** epididymis and **(B)** testis in the S phase of the cell cycle seven days after infection with UPEC. Data were obtained from 4 individual mice. Welch's t-test; mean ± SD was employed for statistical analysis. * $p < 0.05$, ** $p < 0.01$.

**Table S1**

List of antibodies used in this study.

| ANTIBODIES | SOURCE | IDENTIFIER | DILUTION |
|---|---|---|---|
| PerCP/Cy5.5 anti-mouse/human CD11b | Biolegend | Cat#:101228 | 1:100 |
| PE/Cy7 anti-mouse CD11c | Biolegend | Cat#:117318 | 1:100 |
| APC/Cy7 anti-mouse CD45 | Biolegend | Cat#:103116 | 1.100 |
| PE anti-mouse CD64 (FcγRI) | Biolegend | Cat#:139304 | 1:100 |
| Alexa Fluor® 647 anti-mouse CD80 | Biolegend | Cat#:104718 | 1:100 |
| PE anti-mouse CD86 | Biolegend | Cat#:105008 | 1:100 |
| Alexa Fluor® 647 anti-mouse CD115 (CSF-1R) | Biolegend | Cat#:135530 | 1:100 |
| Alexa Fluor® 647 anti-mouse CD206 (MMR) | Biolegend | Cat#:141712 | 1:100 |
| PE/Cy7 anti-mouse CX3CR1 | Biolegend | Cat#:149016 | 1:50 |
| Alexa Fluor® 488 anti-mouse F4/80 | Biolegend | Cat#:123120 | 1:50 |
| Brilliant Violet 510 anti-mouse Ly-6C | Biolegend | Cat#:128033 | 1:100 |
| PE anti-mouse MERTK (Mer) | Biolegend | Cat#:151506 | 1:50 |
| APC anti-mouse MERTK (Mer) | Biolegend | Cat#:151508 | 1:50 |
| Brilliant Violet 510 anti-mouse I-A/I-E | Biolegend | Cat#:107635 | 1:100 |
| Anti-mouse MHC Class II I-Ab | eBioscience | Cat#:17-5320 | 1:100 |
| AlexaFluor 700 anti-mouse CD45 Ab | Biolegend | Cat#: 103128 | 1:100 |
| PerCP anti-mouse CD45.1 | Biolegend | Cat#: 110725 | 1:100 |
| APC anti-mouse CD45.2 | Biolegend | Cat#: 109813 | 1:100 |
| FITC anti-mouse CD45 | Biolegend | Cat#:103107 | 1:100 |
| PE-cy7 anti mouse IA/IE (MHC II) Ab | Biolegend | Cat#:305028 | 1:00 |
| PE-cy5.5 anti-mouse Brdu | Biolegend | Cat#:364110 | 1:50 |
| BV 605 anti-mouse CD64 | Biolegend | Cat#:139323 | 1:50 |
| FITC Rat anti-mouse CD11b | BD bioscience | Cat#:553310 | 1:100 |
| BD Pharmingen™ PE Rat anti-mouse Ly-6G and Ly-6C | BD bioscience | Cat#:553128 | 1:100 |
| APC/Cy7 anti-mouse F4/80 | Biolegend | Cat#:123118 | 1:100 |
| DAPI | BOSTER | Cat#:AR1176 | 1:500 |
| FITC anti-rat IgG2b | Biolegend | Cat#:408205 | 1:100 |
| PE anti-rat IgG2b | Biolegend | Cat#:408213 | 1:100 |
| APC Rat IgG2b, κ Isotype ctrl | Biolegend | Cat#:400611 | 1:100 |
| APC/Cy7 Rat IgG2b, κ Isotype ctrl | Biolegend | Cat#:400623 | 1:100 |
| PerCP/Cy5.5 Rat IgG2b, κ Isotype ctrl | Biolegend | Cat#: 400631 | 1:100 |
| PE/Cy7 Rat IgG2b, κ Isotype ctrl | Biolegend | Cat#: 400617 | 1:100 |
| Alexa Fluor® 594 Rat IgG2b, κ Isotype ctrl | Biolegend | Cat#: 400661 | 1:100 |
| Alexa Fluor® 700 Rat IgG2b, κ Isotype Ctrl | Biolegend | Cat#: 400628 | 1:100 |
| Brilliant Violet 510™ Rat IgG2b, κ Isotype ctrl | Biolegend | Cat#:400645 | 1:100 |