Sex differences in IL-17 contribute to chronicity in male versus female urinary tract infection

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Supplementary Materials:

Figure S1.

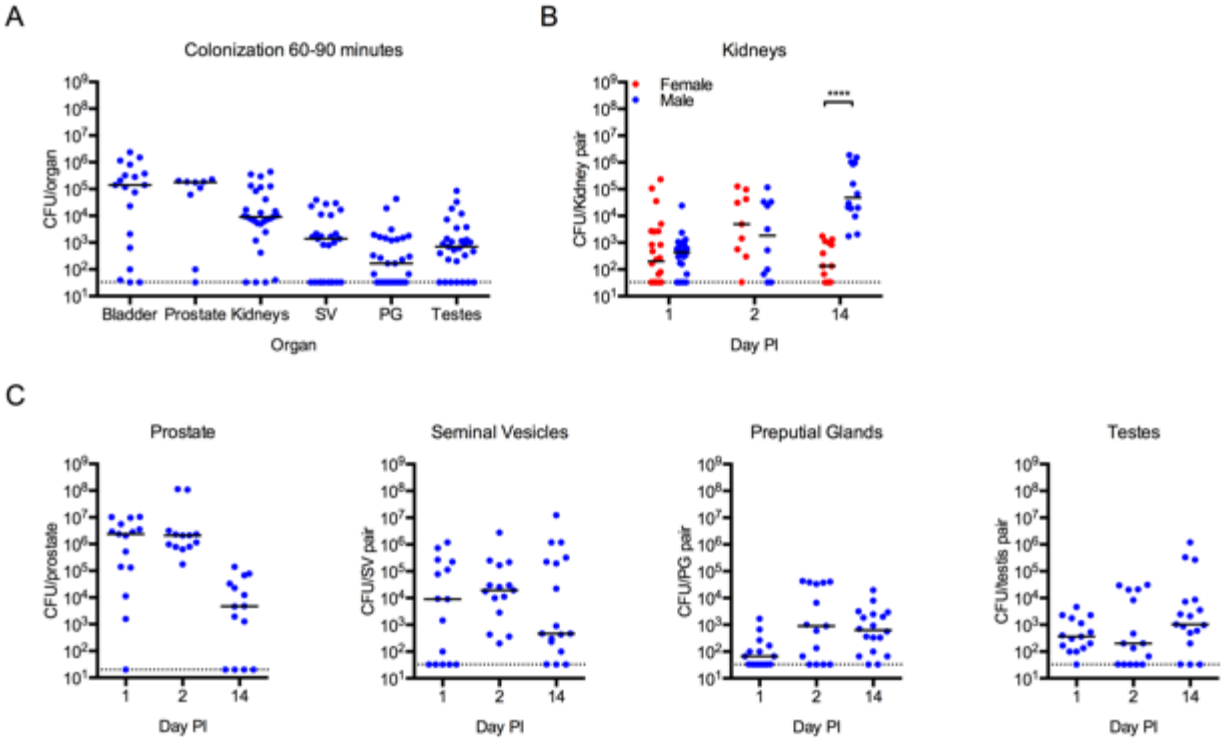

Figure S1. Male mice are colonized by uropathogenic *E. coli* following intravesical instillation *via* the urethra. (**A**) Male C57BL/6J mice were infected with 10^7 colony forming units (CFU) of UPEC strain UTI89-RFP-kan^R and CFU/organ was determined 60-90 minutes post-infection (PI). (**B**) Female and male mice were infected with 10^7 CFU of UPEC strain UTI89-GFP-amp^R or UTI89-RFP-kan^R and CFU/kidney pair was determined at the indicated times PI. (**C**) Graphs depict CFU/organ in male mice infected with 10^7 CFU of UPEC at the indicated times PI. Data are pooled from 2-4 experiments, n=4-10 mice/group in each experiment. Each dot represents one mouse. Dotted lines depict the limit of detection for the assay, 20 or 33 CFU/organ.

Figure S2.

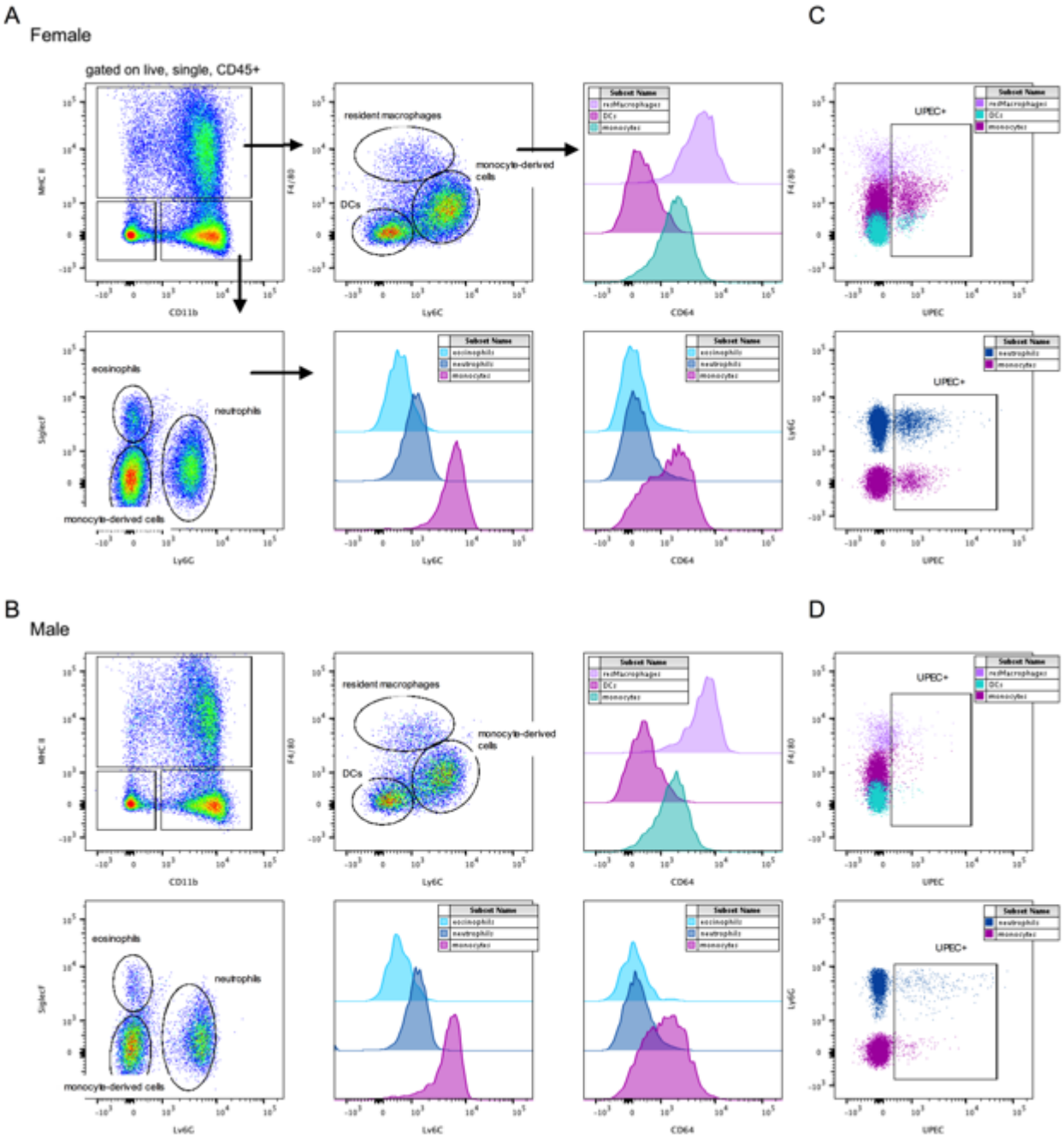

Figure S2. Gating strategy to identify immune cells and UPEC^{+} immune cells. Plots demonstrate the gating strategies used for analyses shown in **Figures 3** and **4**. Representative plots for (**A**) female and (**B**) male mice are shown. Following infection with 10^7 CFU UPEC strain UTI89-RFP-kan^{R}, bladders were digested and immunostained as described in the Methods and (32). Each sample, consisting of a single

bladder, was acquired on a BDFortessa SORP. To identify infiltrating immune cells, samples were gated on $CD45^+$ cells, excluding doublets. Resident macrophages (resMacrophages) were identified as MHC II^+ $CD11b^+$ $F4/80^+$ $CD64^+$ $Ly6C^-$ $Ly6G^-$, dendritic cells (DCs) were MHC II^+ $CD11b^{+/-}$ $CD103^{+/-}$ $CD11c^+$ $F4/80^-$ $CD64^-$ $Ly6C^{+/-}$ $Ly6G^-$, monocyte-derived cells (monocytes) were $CD11b^+$ $Ly6G^-$ $Ly6C^+$ MHC II^- or MHC II^+ $F4/80^{int}$ $CD64^{int}$, neutrophils were identified as $CD11b^+$ $Ly6G^+$ MHC II^- $F4/80^-$ $CD64^-$, eosinophils were $CD11b^+$ $SiglecF^+$ $Ly6G^-$ MHC II^- $F4/80^{int}$ $CD64^-$. (**C-D**) $UPEC^+$ immune cells were identified by population as specified above and then all RFP^+ cells were gated in (**C**) female mice or (**D**) male mice.

Figure S3.

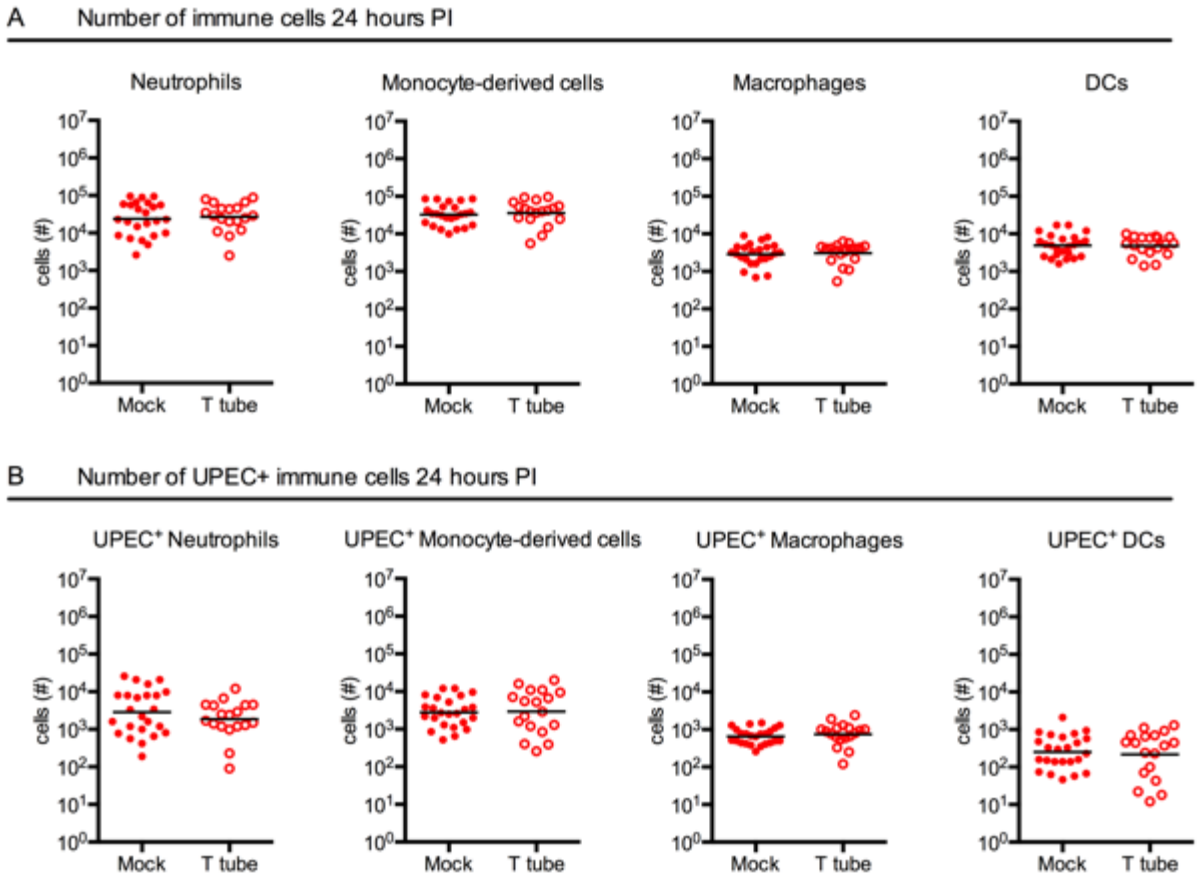

Figure S3. Testosterone treatment in female mice does not alter immune cell infiltration or bacterial phagocytosis. (**A-B**) Female C57BL/6J mice were implanted with empty tubing (Mock) or slow release tubing containing testosterone (T tube) and allowed to recover 1 week before infection with 10^7 CFU UPEC. Graphs show the absolute number of (**A**) infiltrating immune cells and (**B**) $UPEC^+$ immune cells at 24 hours PI. Data are pooled from 4 experiments, n=4-5 mice/group in each experiment, each dot is one mouse, lines are medians. Mann-Whitney test, no statistically significant differences found.

Figure S4.

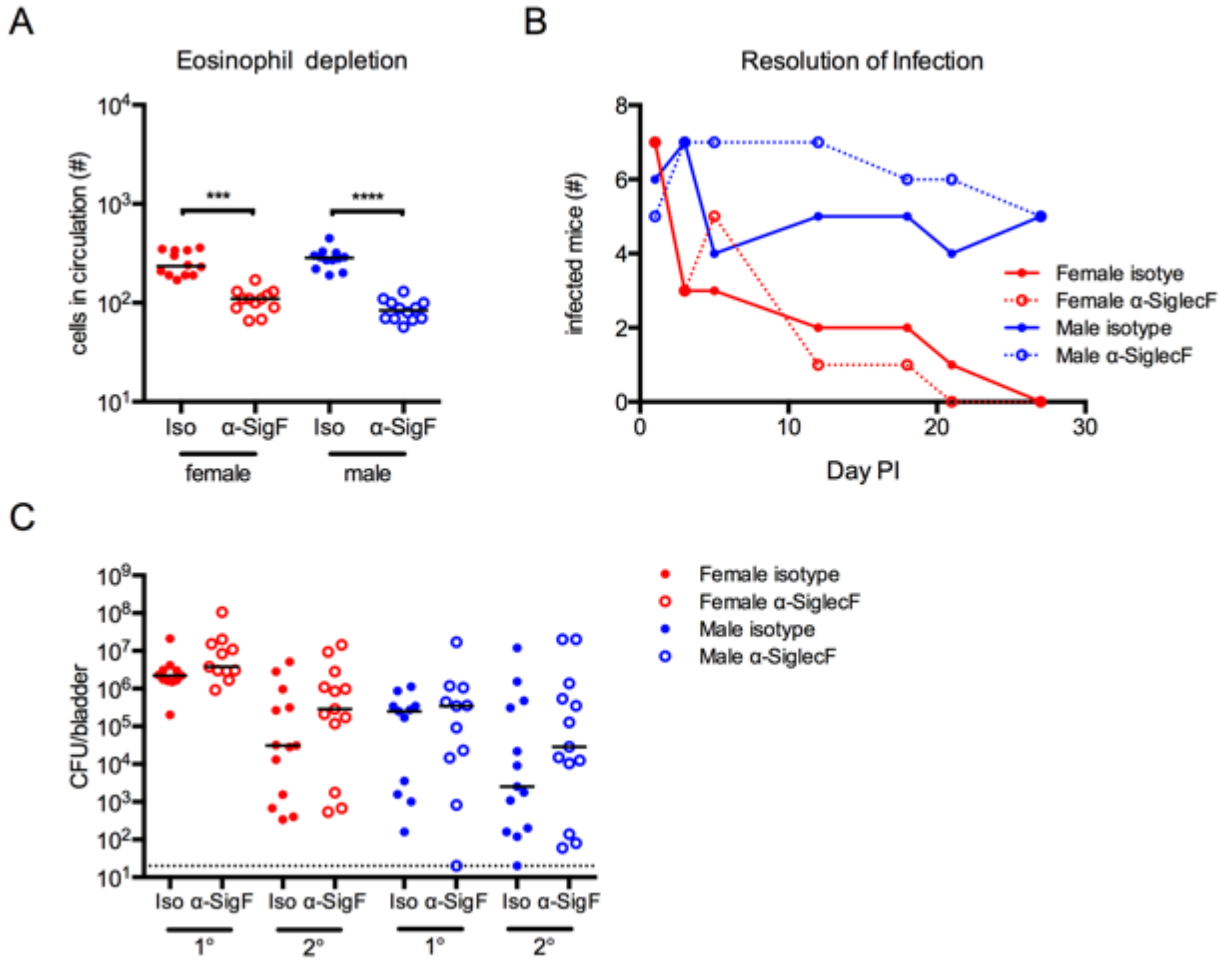

Figure S4. Eosinophils do not mediate resolution of UTI. (**A**-**B**) Female and male C57BL/6J mice were treated with α-SiglecF (α-SigF) antibody or isotype (Iso) control and infected with 10^7 CFU UPEC. Graphs depict the (**A**) number of eosinophils in circulation per μL blood in control and treated mice on the day of infection, (**B**) number of infected mice determined by urine sampling over time, (**C**) CFU at 24 hours post primary (1°) or challenge (2°) infection in the bladder. In **A** and **C**, data are pooled from 2 experiments, n=6-7 mice/group in each experiment, each dot is one mouse, lines are medians. Data in **B** are representative of 3 independent experiments, n=6 mice/group/experiment. *** $p<0.001$, **** $p<0.0001$ Kruskal-Wallis test comparing isotype to α-SiglecF-treated mice within a single sex, with Dunn's post-test to correct for multiple comparisons.

Figure S5.

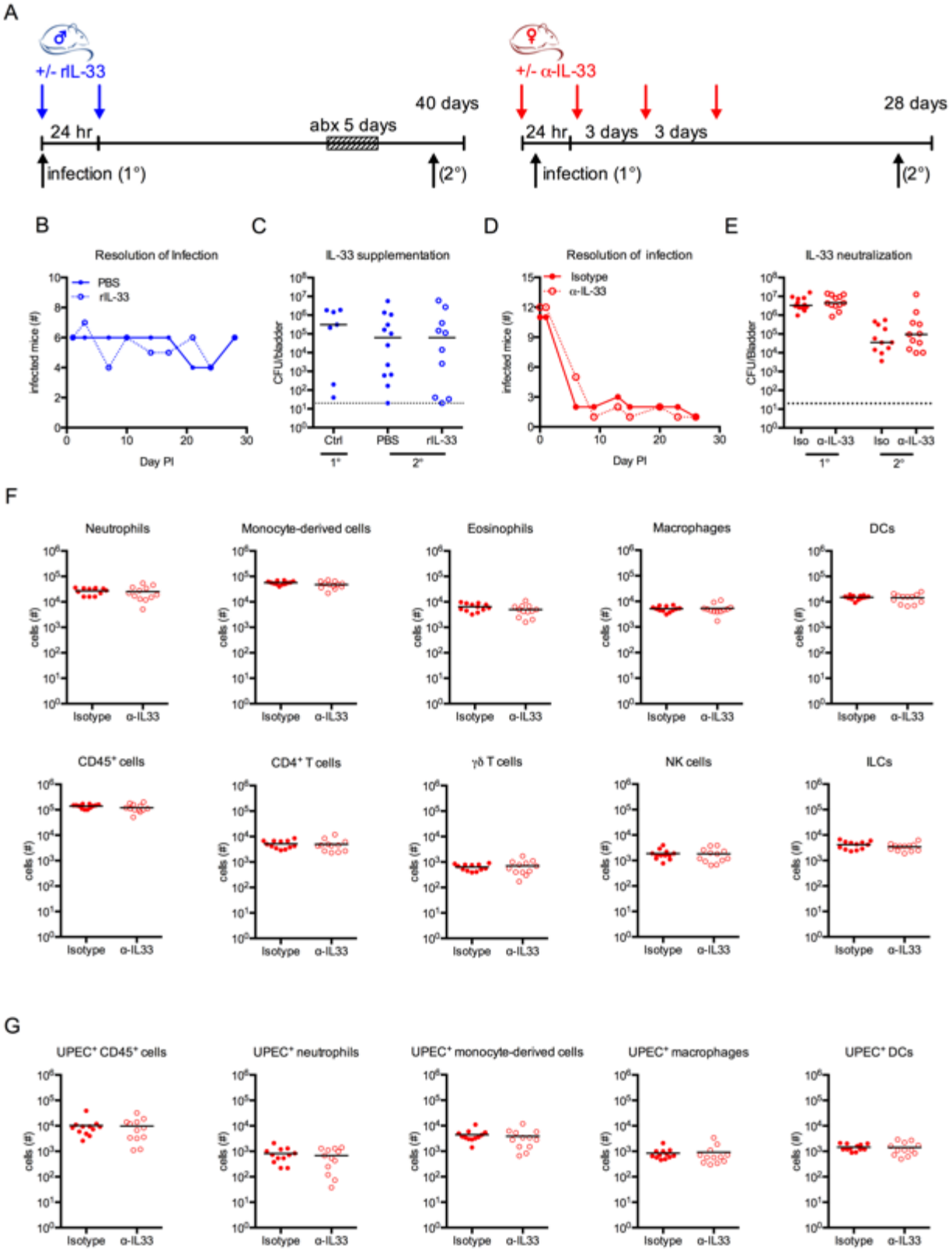
A
+/- rIL-33
24 hr
infection (1°)
abx 5 days
40 days
(2°)
+/- α-IL-33
24 hr
3 days
3 days
28 days
infection (1°)
(2°)
B
Resolution of Infection
PBS
rIL-33
infected mice (#)
Day PI
C
IL-33 supplementation
CFU/bladder
Ctrl
PBS
rIL-33
1°
2°
D
Resolution of infection
Isotype
α-IL-33
infected mice (#)
Day PI
E
IL-33 neutralization
CFU/Bladder
Iso
α-IL-33
Iso
α-IL-33
1°
2°
F
Neutrophils
Monocyte-derived cells
Eosinophils
Macrophages
DCs
CD45+ cells
CD4+ T cells
γδ T cells
NK cells
ILCs
cells (#)
Isotype
α-IL33
G
UPEC+ CD45+ cells
UPEC+ neutrophils
UPEC+ monocyte-derived cells
UPEC+ macrophages
UPEC+ DCs
cells (#)
Isotype
α-IL33

Figure S5. Addition or neutralization of IL-33 does not change the course of infection in male or female mice, respectively. (**A**) Experimental design in which male mice (blue) were supplemented with recombinant IL-33 (rIL-33) or female mice (red) were treated with α-IL-33 neutralizing antibody. All mice were infected with 10^7 CFU UPEC and bacteriuria was assessed over time. (**B-G**) Graphs show (**B**) the number of infected male mice over time and (**C**) CFU at 24 hours post primary (1°) or challenge (2°) infection in the bladder, in PBS or rIL-33 treated male mice; (**D**) the number of infected female mice over time and (**E**) CFU at 24 hours post primary (1°) or challenge (2°) infection in the bladder, in isotype or α-IL-33 treated female mice; and the absolute number of the (**F**) indicated immune cell populations and (**G**) $UPEC^+$ immune cell populations in bladders 24 hours PI in isotype or α-IL-33 treated female mice. Data in **B** and **D** are representative experiments of 2 independent experiments, n=6 mice/group/experiment. In **C, E-G**, data are pooled from 2 experiments, n=4-6 mice/group in each experiment, each dot is one mouse, lines are medians. Mann-Whitney test, no statistically significant differences found.

Figure S6.

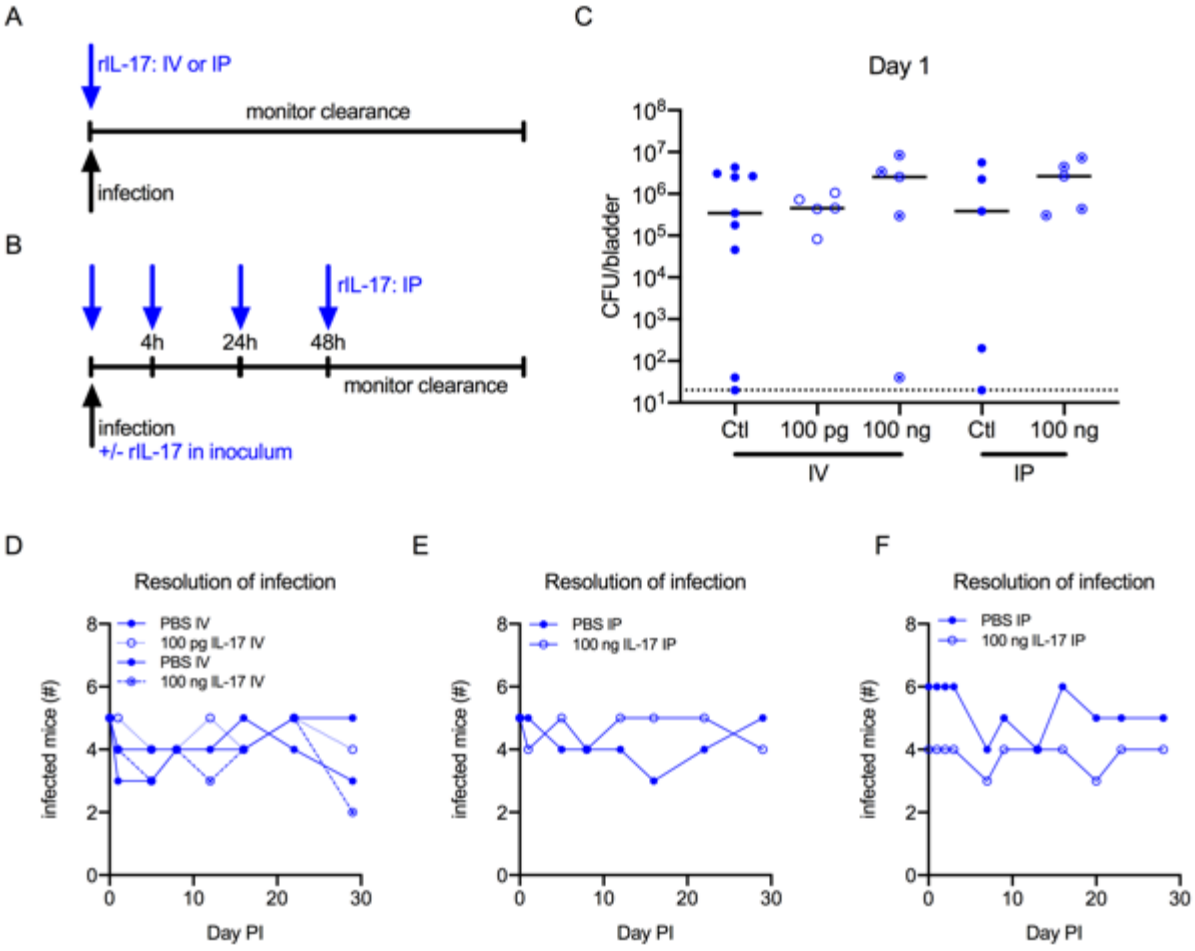

Figure S6. Recombinant IL-17 does not change the course of infection in male mice. (**A-B**) Experimental design in which male mice were supplemented with recombinant IL-17 (**A**) one time or (**B**) four times during infection. All mice were infected with 10^7 CFU UPEC and bacteriuria was assessed over time. (**C**) Graph shows CFU at 24 hours PI in the bladder, in male mice treated with PBS or rIL-17- one time. (**D-F**) Graphs show the number of infected male mice over time following (**D**) single intraveneous, (**E**) single intraperitoneal, or (**F**) multiple intraperitoneal injections of IL-17. Each graph (**C-F**) depicts an independent experiment, n=4-6 mice/group/experiment. In **C**, each dot is one mouse, lines are medians, Ctl IV shows the control groups for 100 pg and 100 ng pooled together. Mann-Whitney test, no statistically significant differences.

Table S1. Differentially expressed genes in *E. coli* stimulated donor blood. Differentially expressed genes (DEG) following whole blood stimulation of 300 healthy female and 300 healthy male donors aged 20-49 years. Table shows p-values and q-values as determined by T-test and FDR T-test respectively, and fold change differences (values >1 reflect higher expression in females), between female and male whole blood stimulated with heat killed *E. coli*.

Supplemental Table 2: Antibodies used in this study

Molecule	Clone	Vendor
CD45	30-F11	BD Bioscience
CD64	X54-5/7.1	BD Bioscience
CD103	M290	BD Bioscience
CD11b	M1/70	BD Bioscience
CD11c	N418	eBioscience
F4/80	CI:A3-1	AbD Serotec
Ly6C and Ly6G (Gr-1 antibody)	RB6-8C5	BD Bioscience
Ly6G	1A8	BD Bioscience
Ly6C	AL-21	BD Bioscience
MHC-II (I-A/I-E)	M5 or 114.15.2	eBioscience
SiglecF	E50-2440	BD Bioscience
CD3	145-2C11	BD Bioscience
CD4	RM4-5, GK1.5	BD Bioscience
CD25	PC61	BD Bioscience
CD90.2	53.2.1	ebioscience
IL-4Rα (CD124)	mIL4R-M1	BD Bioscience
TCR γ/δ	GL-3	Biolegend
NK1.1	PK136	BD Bioscience