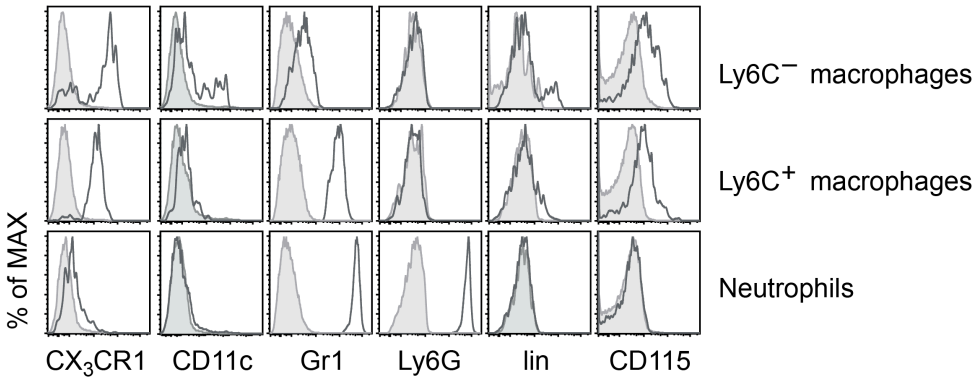
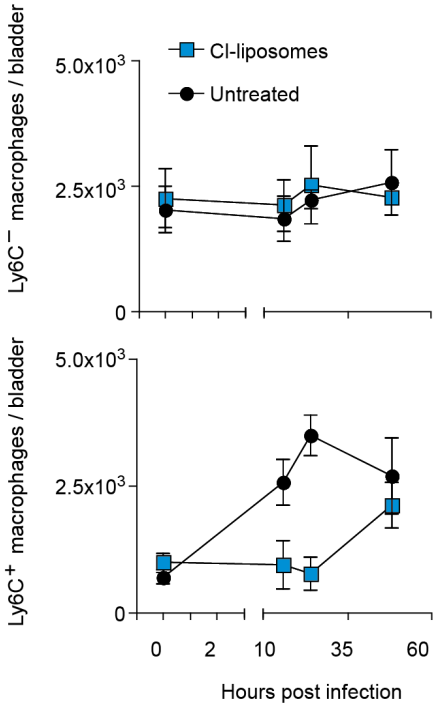
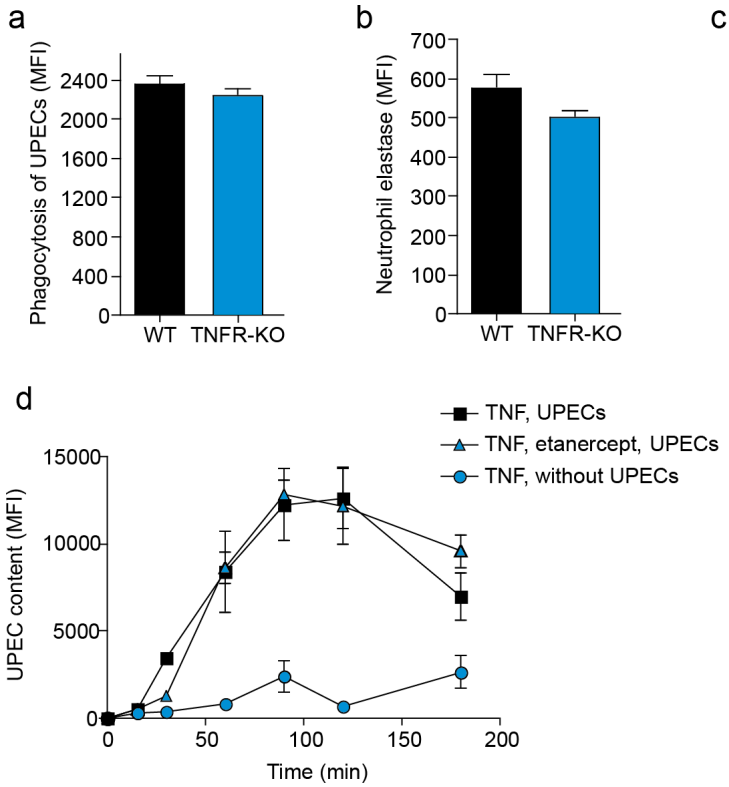


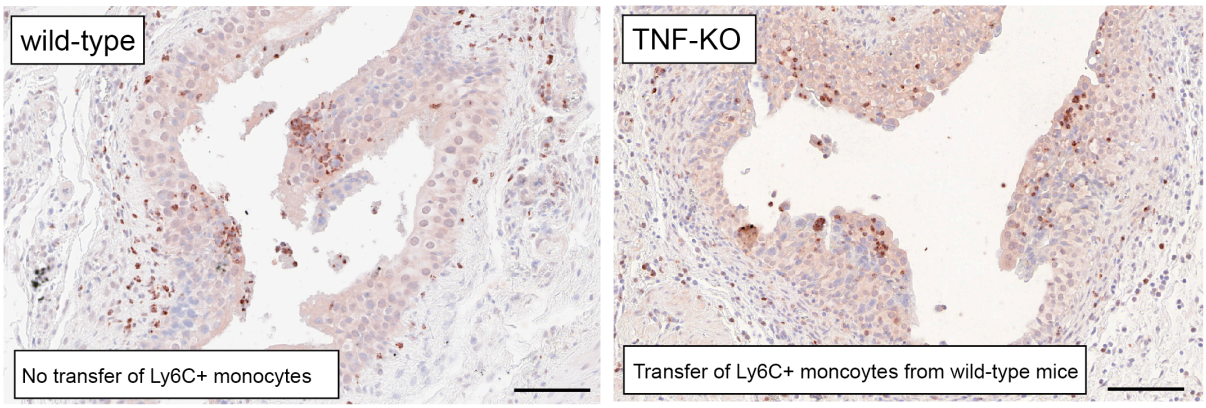
Supplementary Figure 1: Phenotype of neutrophils, Ly6C$^-$ and Ly6C$^+$ macrophages. Flow cytometry analysis of bladder homogenates of infected CX$_3$CR1$^{+/GFP}$ mice. Neutrophils, Ly6C$^+$ and Ly6C$^-$ macrophages were distinguished by their expression of Ly6C and F4/80 (**Fig. 1a**) and analyzed for expression of CX$_3$CR1, CD11c, Gr1, Ly6G, lin (CD3, B220, NK1.1) and CD115.



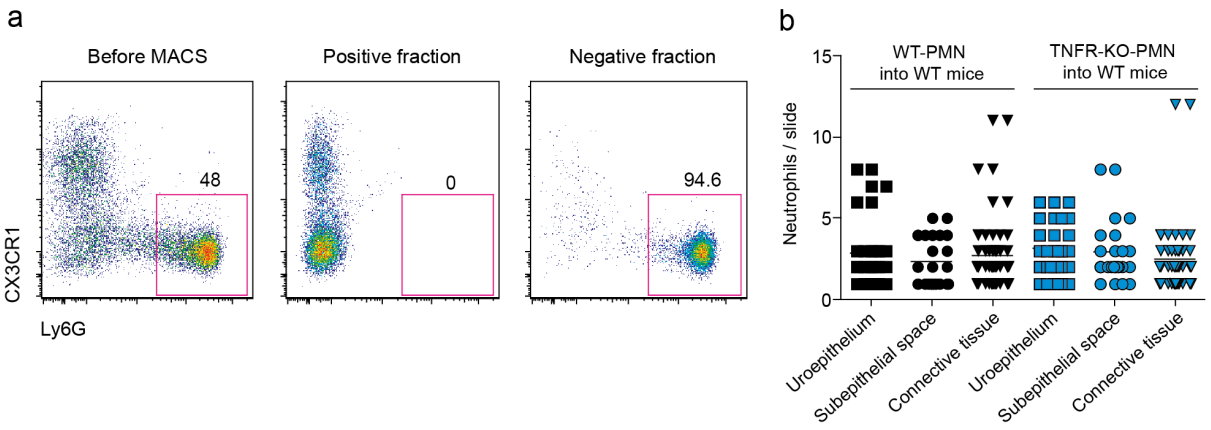
Supplementary Figure 2: Only Ly6C$^+$ macrophages are sensitive to clodronate-liposome-mediated deletion. Numbers of Ly6C$^-$ (top) and Ly6C$^+$ (bottom) macrophages determined at different time points by flow-cytometry in mice treated intravenously with clodronate-liposomes 6 hours prior to infection. Data represent three independent experiments with five mice per group.



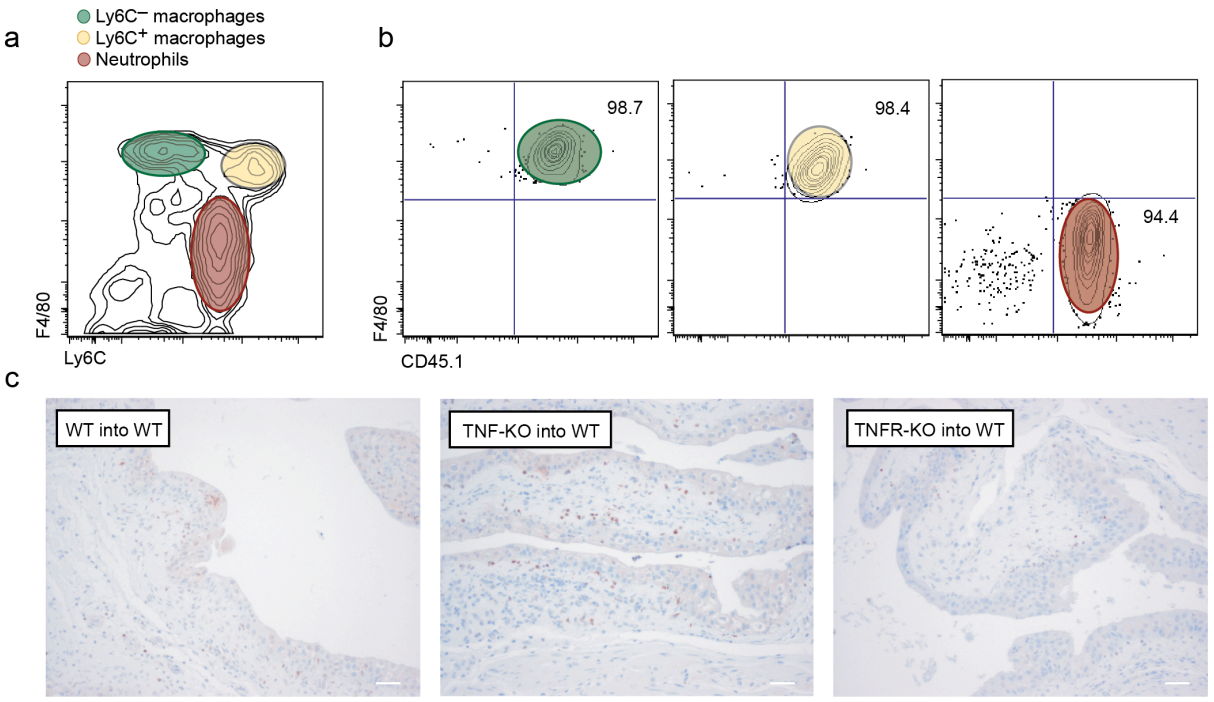
Supplementary Figure 3: Antibacterial effector function of neutrophils is not influenced by TNF. **a**, Flow-cytometric quantitation of phagocytosis of UPECs expressing recombinant GFP by bone marrow-derived neutrophils from wild-type (WT) or TNFR-KO mice. **b,c**, Elastase (**b**) and MPO (**c**) content in neutrophils from infected WT or TNFR-KO mice, determined by flow cytometry 6 hours after infection. **d**, Bacterial uptake by bone marrow-derived neutrophils after incubation with GFP-expressing UPECs in the presence of 10 ng/ml TNF (rectangles), TNF and 20 µg/ml etanercept (triangles) or left untreated (circles), determined by flow cytometry for GFP content. Extracellular bacteria were killed with 100 µg/ml gentamycin after 100 minutes to prevent interference with the assay for intracellular bactericidal activity. Data are means ± s.e.m. and represent two to three independent experiments with three mice per group.



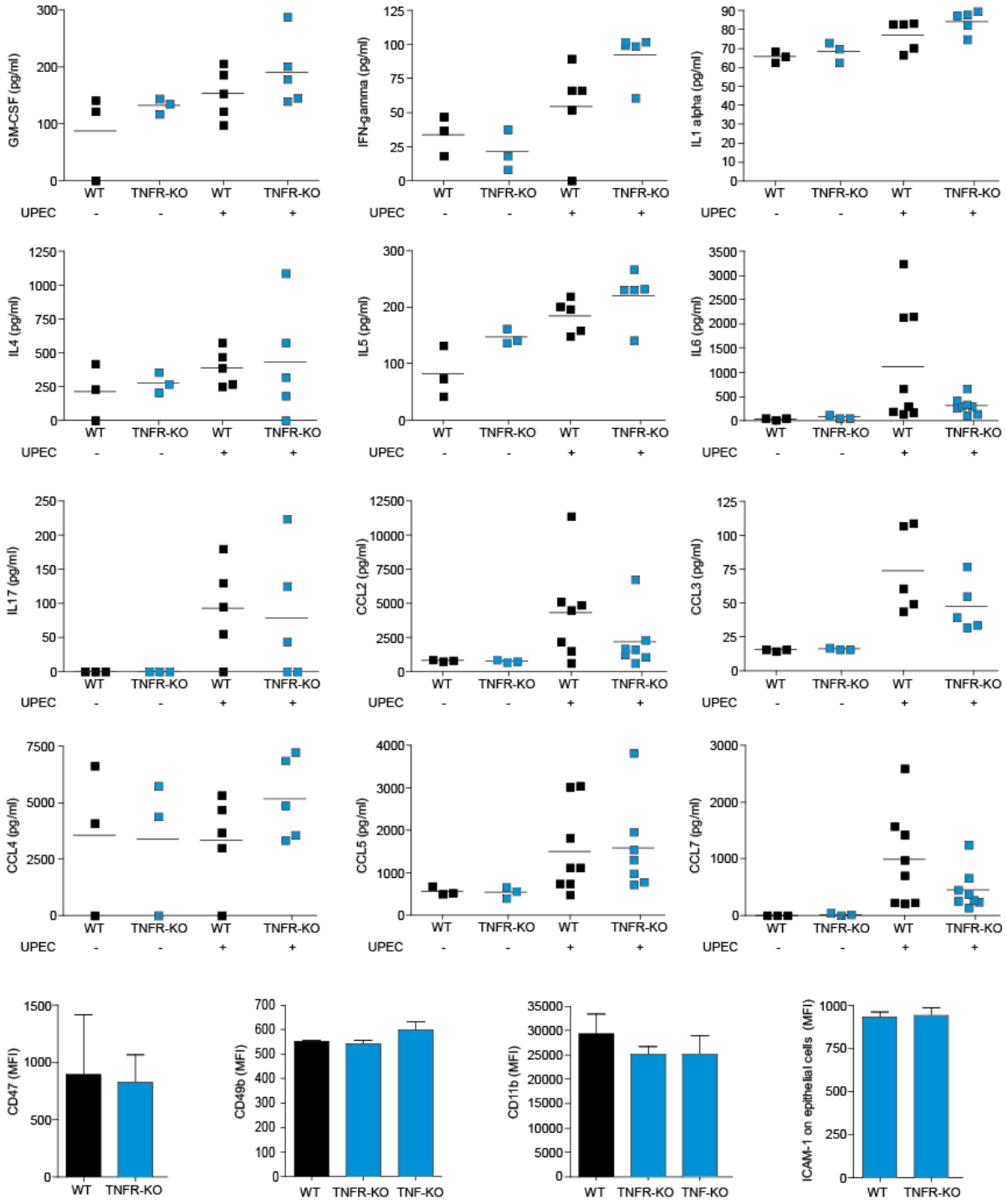
Supplementary Figure 4: Transfer of Ly6C$^+$ monocytes into TNF-deficient mice restores transepithelial neutrophil migration. Paraffin sections one day after infection of wild-type or TNF-KO mice that had received Ly6C$^+$ bone marrow monocytes from wild-type mice 10 minutes prior to infection. The bar graph indicates 100µm. Data are means ± s.e.m. and represent two independent experiments with three mice per group. Where are the bar graphs ?



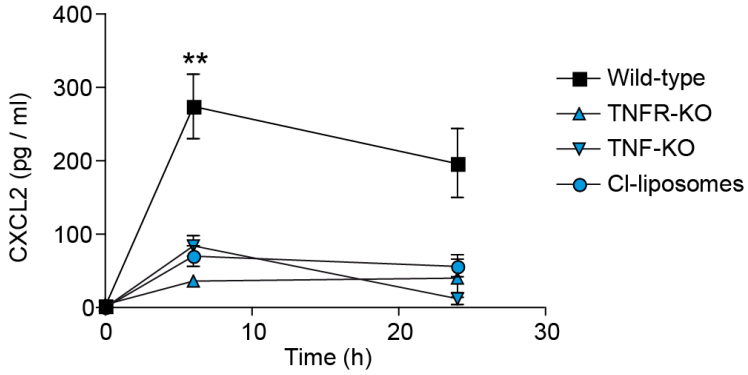
Supplementary Figure 5: TNF acts in trans to enable transepithelial neutrophil migration. Neutrophils from the bone marrow of wild-type and TNFR-KO mice were labelled with CFSE and adoptively transferred into wild-type animals. One day after transfer CFSE$^+$ neutrophils were detected by Ly6G staining in bladder cryosections. Data are means ± s.e.m. and represent three independent experiments in groups of five mice.



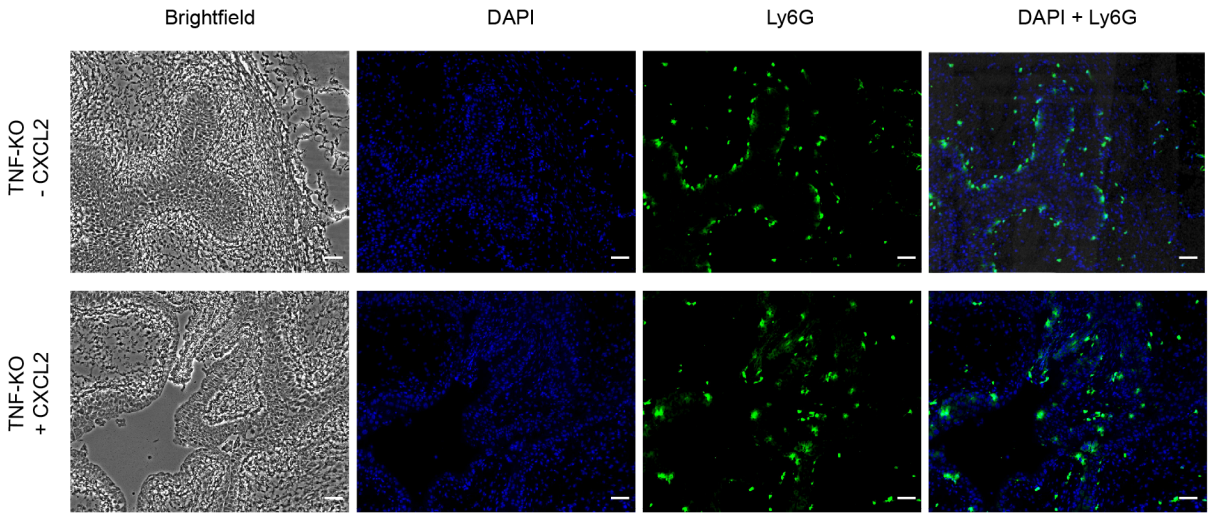
Supplementary Figure 6: TNF by bone marrow-derived cells is essential for transepithelial migration of neutrophils. a, **b**, Efficient reconstitution of CD45.1 donor neutrophils, Ly6C$^+$ and Ly6C$^-$ macrophages within the bladder 8 (**a**) and 12 (**b**) weeks after irradiation of CD45.2 wild-type mice and transfer of CD45.1 bone marrow from wild-type animals. **c**, Histological sections of one day infected bone marrow chimeric mice expressing TNF or TNFR in all cells (WT into WT) or only in non-hematopoietic cells (TNFKO into WT, TNFR-KO into WT, respectively) by Ly6G. Quantification is shown in Fig. 3e.



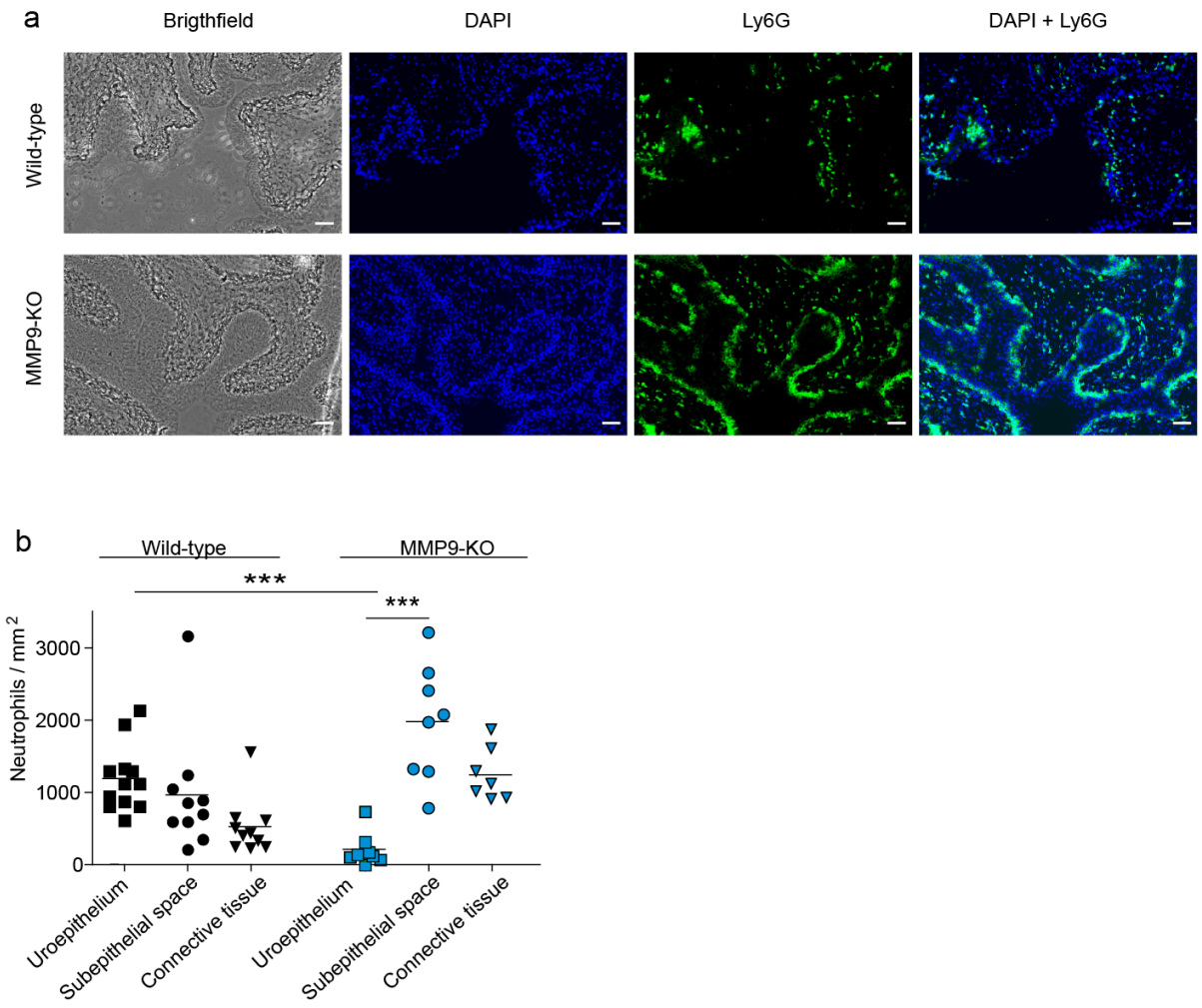
Supplementary Figure 7: Expression of molecules in the infected bladder. a, Chemokine and cytokine levels in homogenates from infected (UPEC +) and uninfected (UPEC +) wild-type (WT) and TNFR-KO bladders, determined by FlowCytomix Multiplex assay 6 hours after infection. **b**, Expression of CD47, CD11b, CD49b and ICAM-1 in the infected bladder of WT, TNF-KO and TNFR-KO mice. Data are means ± s.e.m. and represent three independent experiments with 3-4 mice per group. **a** and **b** are not indicated.



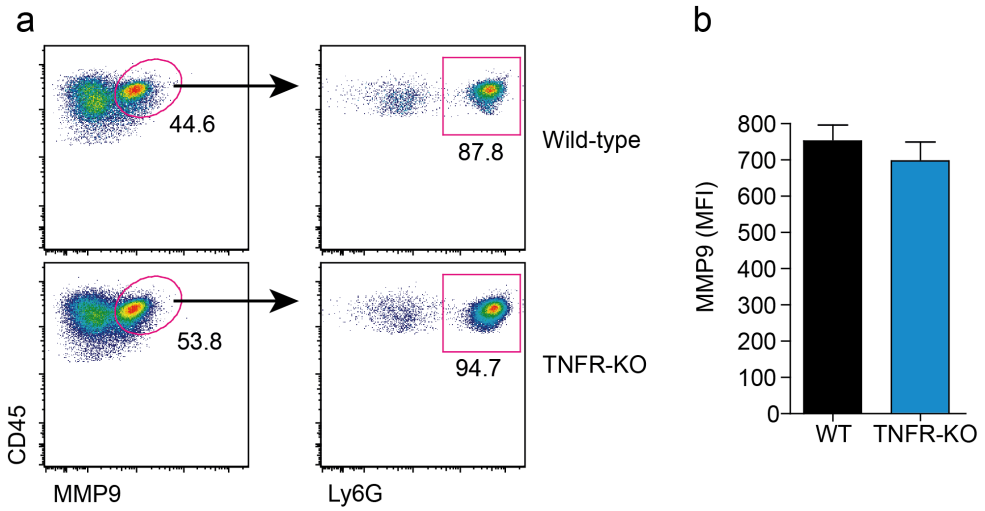
Supplementary Figure 8: Lack of CXCL2 release in TNF- and TNFR KO mice and after depleting Ly6C$^+$ macrophages. CXCL2 expression was determined by ELISA in bladder homogenates of wild-type, TNFR-KO, TNF-KO and clodronate-liposome-treated animals (Cl-liposomes) by ELISA 6 and 24 hours after infection.



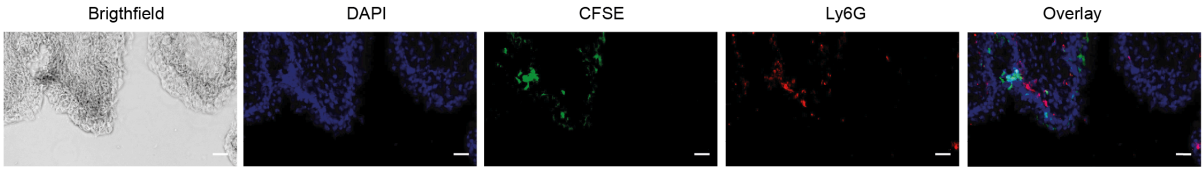
Supplementary Figure 9: TNF-dependent CXCL2 enables transepithelial migration of neutrophils. Position of neutrophils revealed by green Ly6G staining of bladder sections from infected TNF-KO mice transurethrally injected after 24 and 27 hours with CXCL2 (+CXCL2; lower row) or not injected (-CXCL2; upper row) and mice were sacrificed 28 hours post infection. Depicted are extracted bright field and fluorescence images (DAPI in blue, Ly6G in green) of the image shown in Fig. 4e.



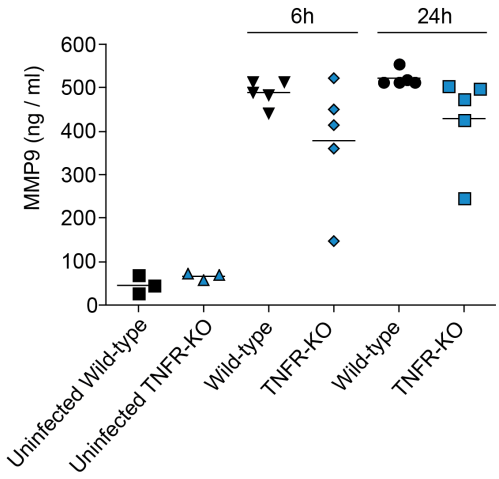
Supplementary Figure 10: MMP-9 is required for neutrophil migration into the uroepithelium. a, Position of neutrophils revealed by green Ly6G staining of bladder sections from wild-type (upper row) and MMP-9-KO mice (lower row) 24 hours after infection, stained with Ly6G (green) and nuclear DAPI counterstaining (blue). **b**, Quantitative analysis of experiments as depicted in panel (**a**).



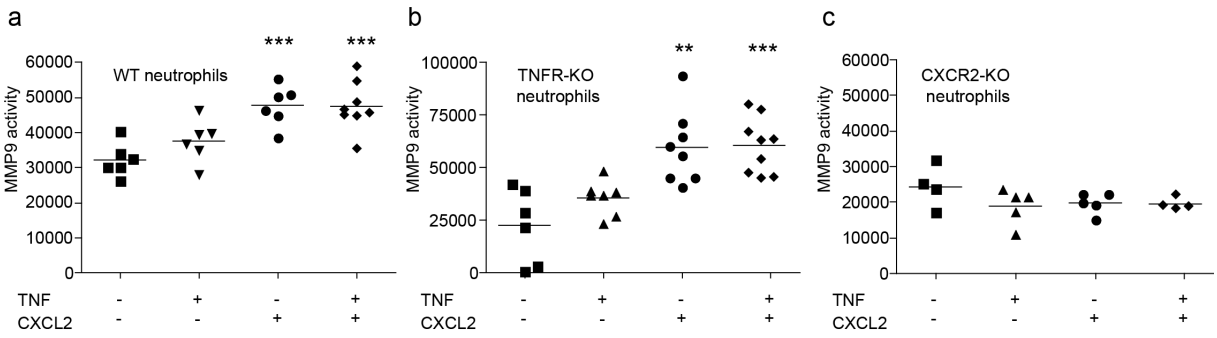
Supplementary Figure 11: MMP-9 is mainly expressed by neutrophils and is independent of TNFR. a, Coexpression of the neutrophil marker Ly6G in MMP9^{+} cells in infected wild-type and TNFR-KO mice 6 hours after infection by flow-cytometry. **b**, MMP9 expression levels in Ly6G^{+} neutrophils determined by flow-cytometry (MFI=mean fluorescence intensity) 6 hours after infection.



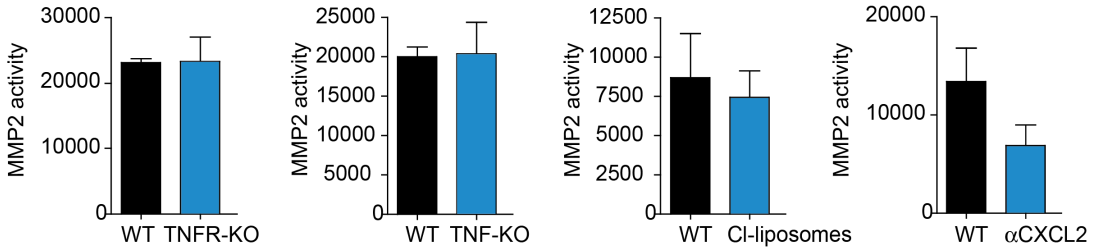
Supplementary Figure 12: MMP-9-competent neutrophils migrate into the uroepithelium in MMP-9-KO mice. Representative staining of Ly6G-AlexaFluor568-stained neutrophils in cryosections of bladders of infected MMP-9 mice that had been injected with CFSE-labelled bone marrow from wild-type mice.



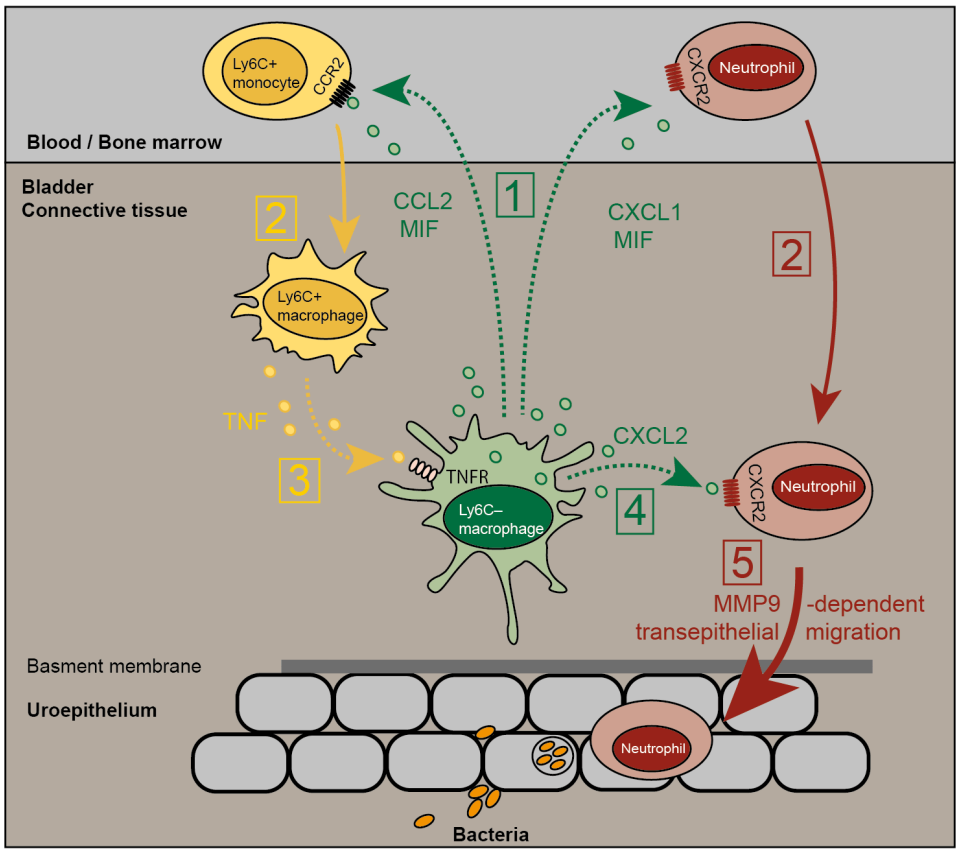
Supplementary Figure 13: Expression of MMP-9 in homogenates is not regulated by TNF. MMP-9 protein expression in bladder homogenates from uninfected and infected WT and TNFR-KO mice, determined by ELISA 6 and 24 hours after infection. Data are means ± s.e.m. and represent two independent experiments with 3-5 mice per group.



Supplementary Figure 14: MMP-9 levels are increased by CXCL2. Supernatants were obtained from cell cultures containing bone marrow-derived neutrophils from WT (**a**), TNFR-KO (**b**) and CXCR2-KO (**c**) mice. Neutrophils were stimulated with TNF and/or CXCL2 as indicated or left untreated and MMP-9 levels were determined by zymography. Data are means ± s.e.m. and represent four independent experiments with four (**a**) and three (**b, c**) mice per group.



Supplementary Figure 15: MMP-2 levels are influenced neither by TNF nor CXCL2. MMP-2 in bladder homogenates from WT, TNFR-KO, TNF-KO, macrophage-depleted (Cl-liposomes) and anti-CXCL2 treated (αCXCL2) mice, determined by zymography 6 hours after infection. Data are means ± s.e.m. and represent four independent experiments in four (**a**), three (**b, c**) groups of 3-5 mice.



Supplementary Figure 16: Graphical summary of the sequence of events. 1) Resident Ly6C^{-} macrophages sensed the infection and produced chemokines including CXCL1, MIF and CCL2, which recruited neutrophils and Ly6C^{+} macrophages, 2) recruited Ly6C^{+} macrophages produced TNF, 3) TNF induced CXCL2 production by Ly6C^{-} macrophages, 4) CXCL2 caused MMP9 activation in neutrophils, which allowed them to cross the epithelial basement membrane in order to combat the infection.