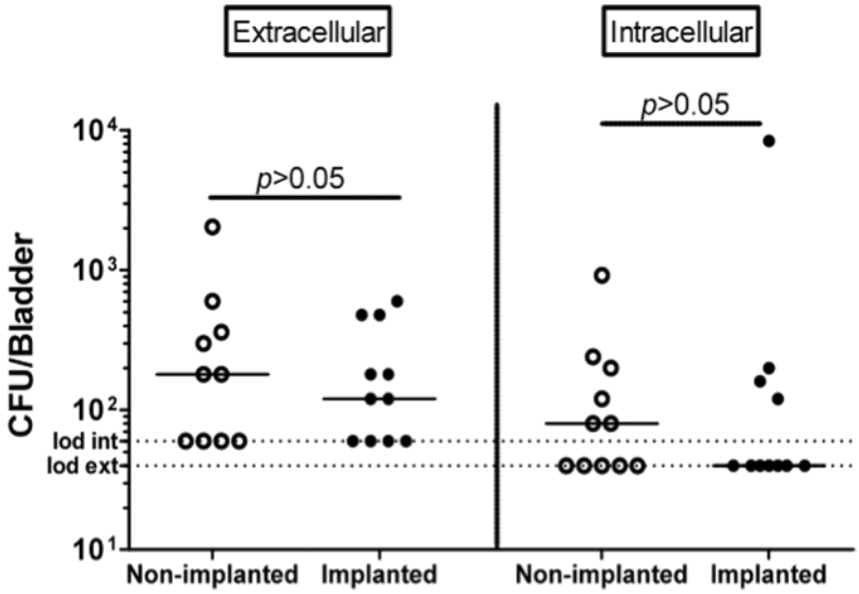
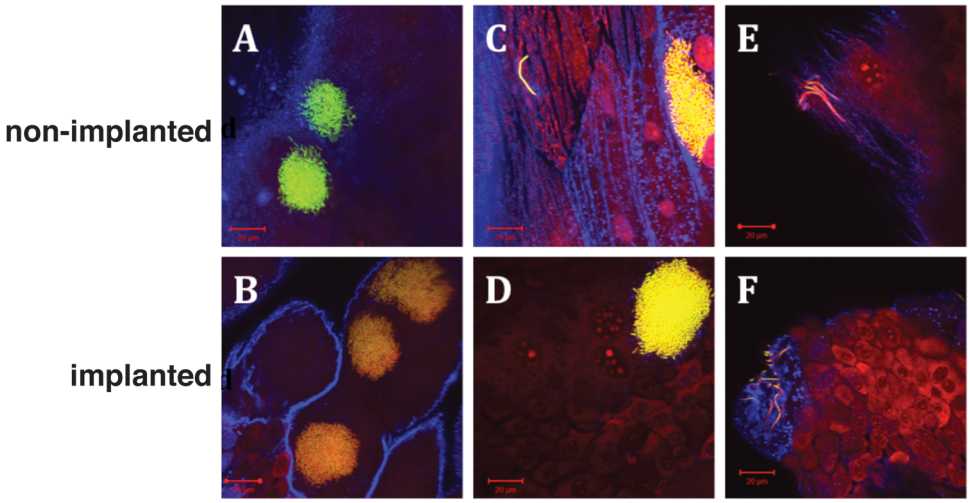
Supplemental materials

S1. No defect in UTI89 invasion following implantation



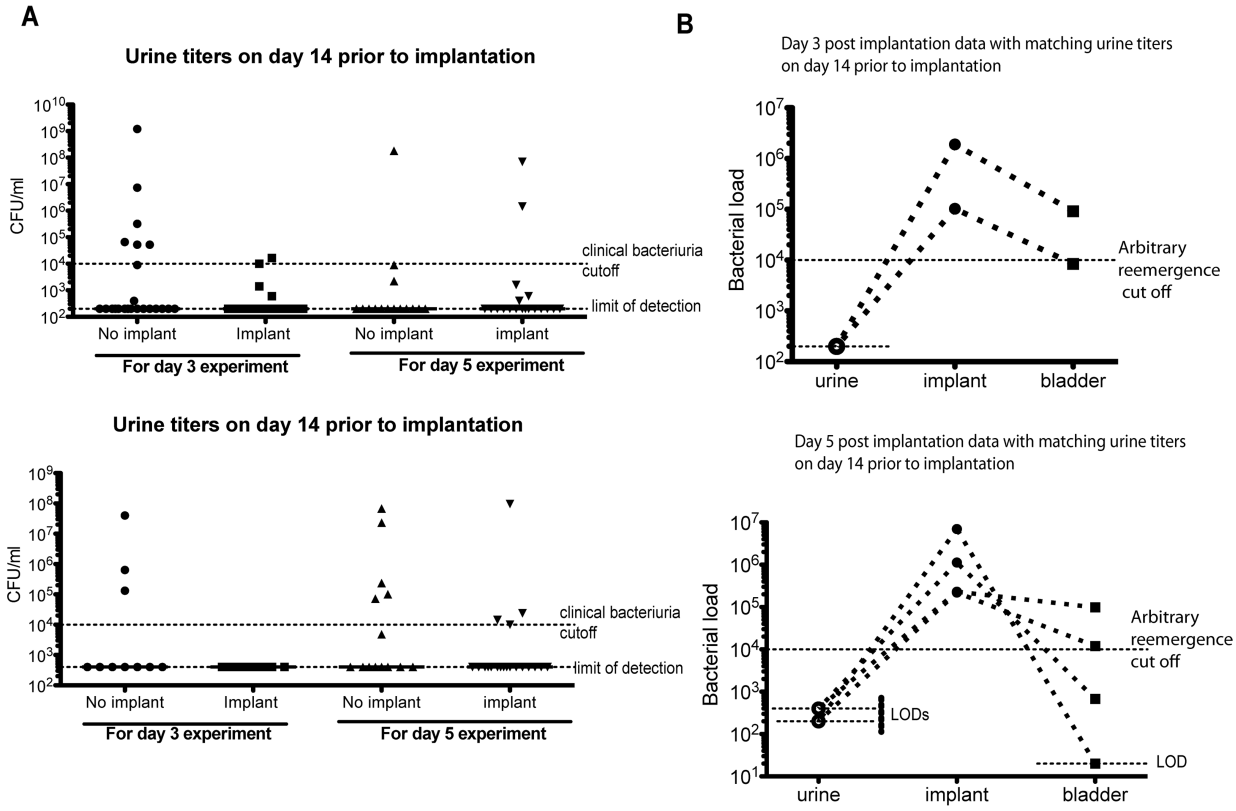
Graph represents bacterial titers from homogenized bladders from non-implanted (○) or implanted (●) animals infected with UTI89 3hpi following gentamicin protection assay. Horizontal dashed lines represent the limit of detection (lod) for viable bacteria (Int=intracellular, Ext=Extracellular). Each symbol represents a mouse from two independent experiments with n=5/condition. The horizontal bars represent the median of each dataset; *p* value by the Mann Whitney U test.

S2. IBC and filamentation occur following urinary catheterization



Representative CLSM images of whole bladders from non-implanted and implanted animals infected with UTI89 ectopically expressing GFP (Green), stained with DNA dye SYTO83 (Red) and Alexa-fluor 633-conjugate of WGA (Blue) reveal the presence of multiple IBCs within single umbrella cells **(A-B)**, that unlike non-implanted bladders **(C),** the underlying epithelium is exposed following urinary catheterization **(D-F)**, depict the absence of bacterial colonization of the exposed underlying epithelium in implanted animals **(D-F)**, and the presence of filamenting bacteria in umbrella cells **(E-F)**. Scale bar =20μm

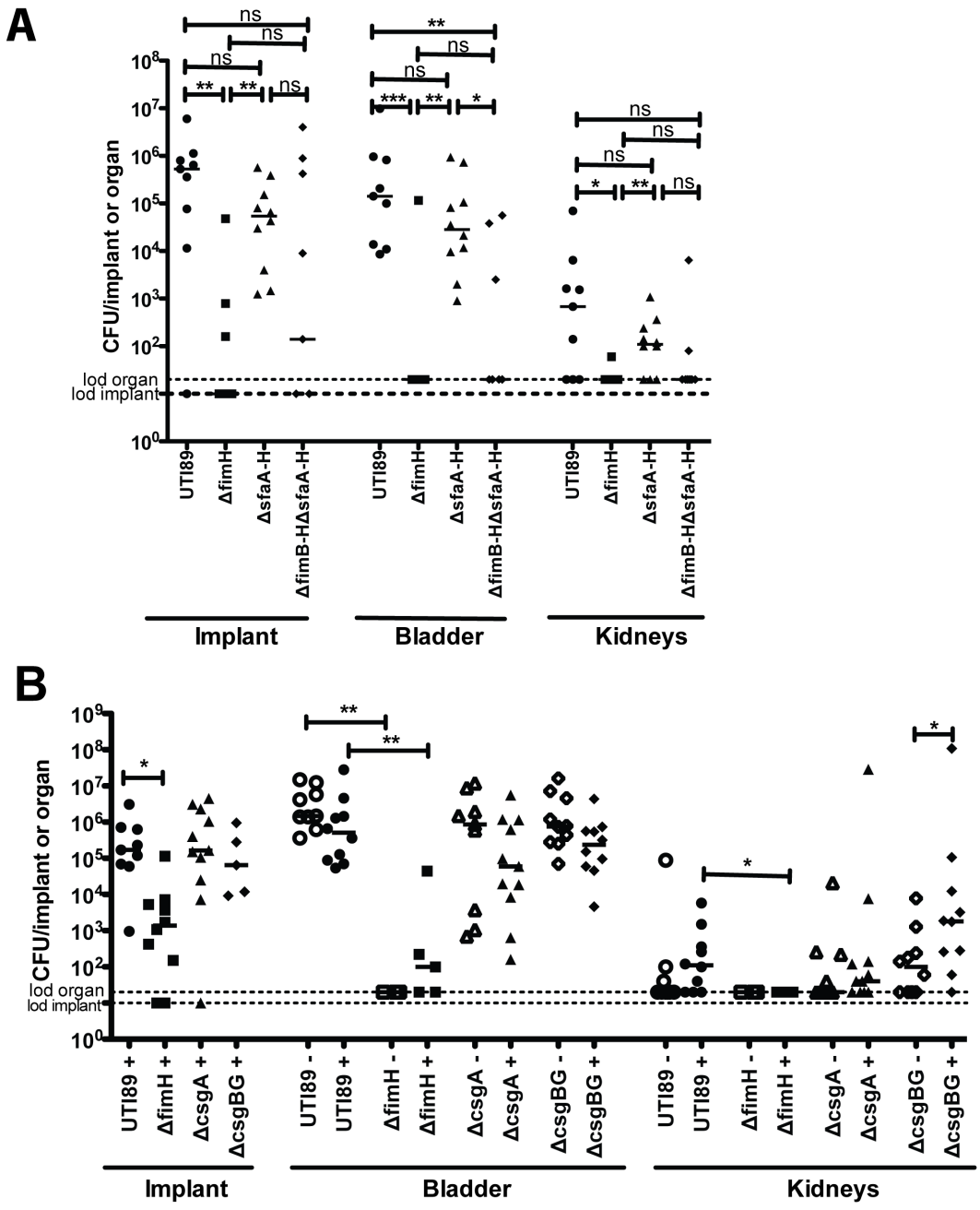
S3. UPEC reemerges from reservoirs following urinary implantation in abacteriuric animals.



(A) Graphs represent bacterial titers from urine collected from non-implanted animals on day 14 post infection with marked strain UTI89HK::GFP from two independent experiments. “implant” group were implanted either for 3 days or 5 days and “no implant” group remained without implant. Only animals with urine titers at the limit of detection (LOD) for each experiment (200 or 400 CFU/ml) were considered abacteriuric and used for further analyses. **(B)** Graphs represent urine titers obtained on day 14 from non-implanted animals matched with the corresponding bacterial titers recovered from implants and bladders on day 3 (top) and 5 (bottom) post implantation of the same animals. Only implanted animals with reemerging infections (bacterial loads

≥104CFU/implant or bladder) are displayed. None of the abacteriuric non-implanted animals had reemerging infections (Fig. 2 in the main manuscript). LOD for bladders=20CFU/ml

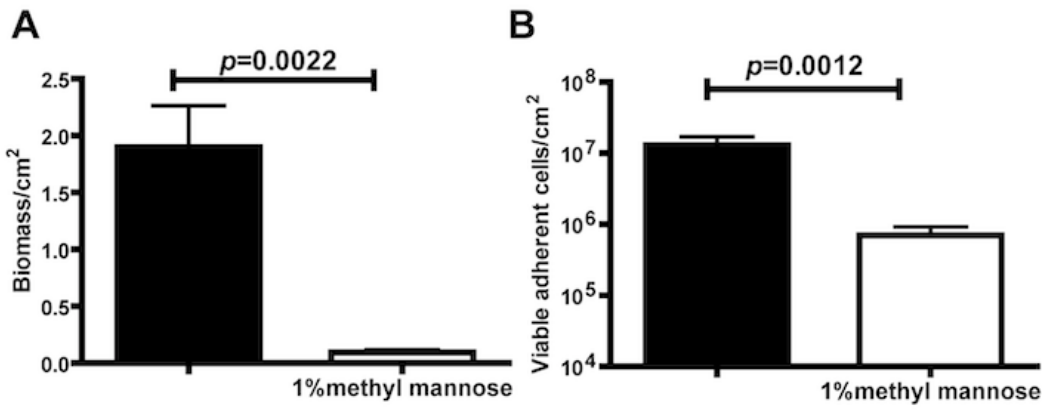
S4. S pili and curli are not critical for UPEC virulence following urinary catheterization



Graphs represent bacterial titers in log scale recovered at 24hpi from implants, homogenized bladders and kidneys of **(A)** implanted animals infected with either UTI89

(●) or UTI89 mutant strains deficient in type 1 pili, Δ*fimH* (■), S pili Δ*sfaA-H*(▲), both type 1 and S pili Δ*sfaA-H*Δ*fimB-H*(♦) and **(B)** non-implanted (open symbols) or implanted (closed symbols) animals infected with UTI89 (○,●), Δ*fimH* (□, ■) or UTI89 mutant strains deficient in curli components Δ*csgA* (Δ,▲) and Δ*csgB*Δ*csgG* (♦,◊). Horizontal dashed lines represent the limit of detection for viable bacteria. Each symbol represents a mouse from at least two independent experiments with n=5/group. The horizontal bars represent the median of each dataset; *p<0.05 and ***p<0.005 **p<0.0005 by the Mann Whitney U test.

S5. Methyl mannose inhibits UPEC biofilm in human urine



Graphs represent crystal violet based quantification **(A)** and CFU enumeration in logarithmic scale (log scale) **(B)** of 24h old UTI89 biofilms in human urine with or without 1% methyl mannose under flow on silicone tubings at 37ºC indicating that methyl mannose prevents UPEC biofilm formation. Bars represent mean of three independent experiments, error bars indicate standard error of the mean (SEM). *p* values from Mann Whitney U test.