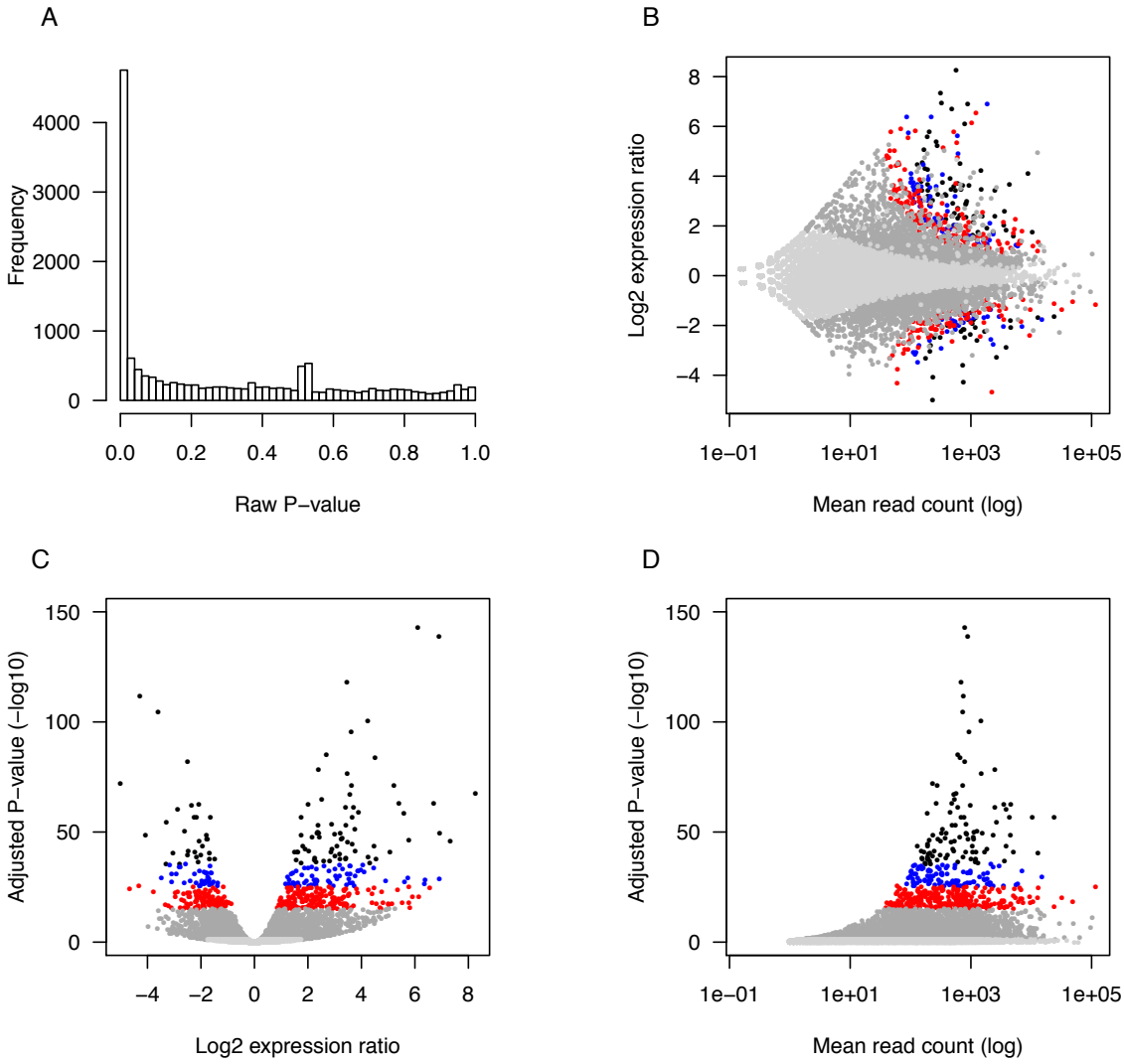
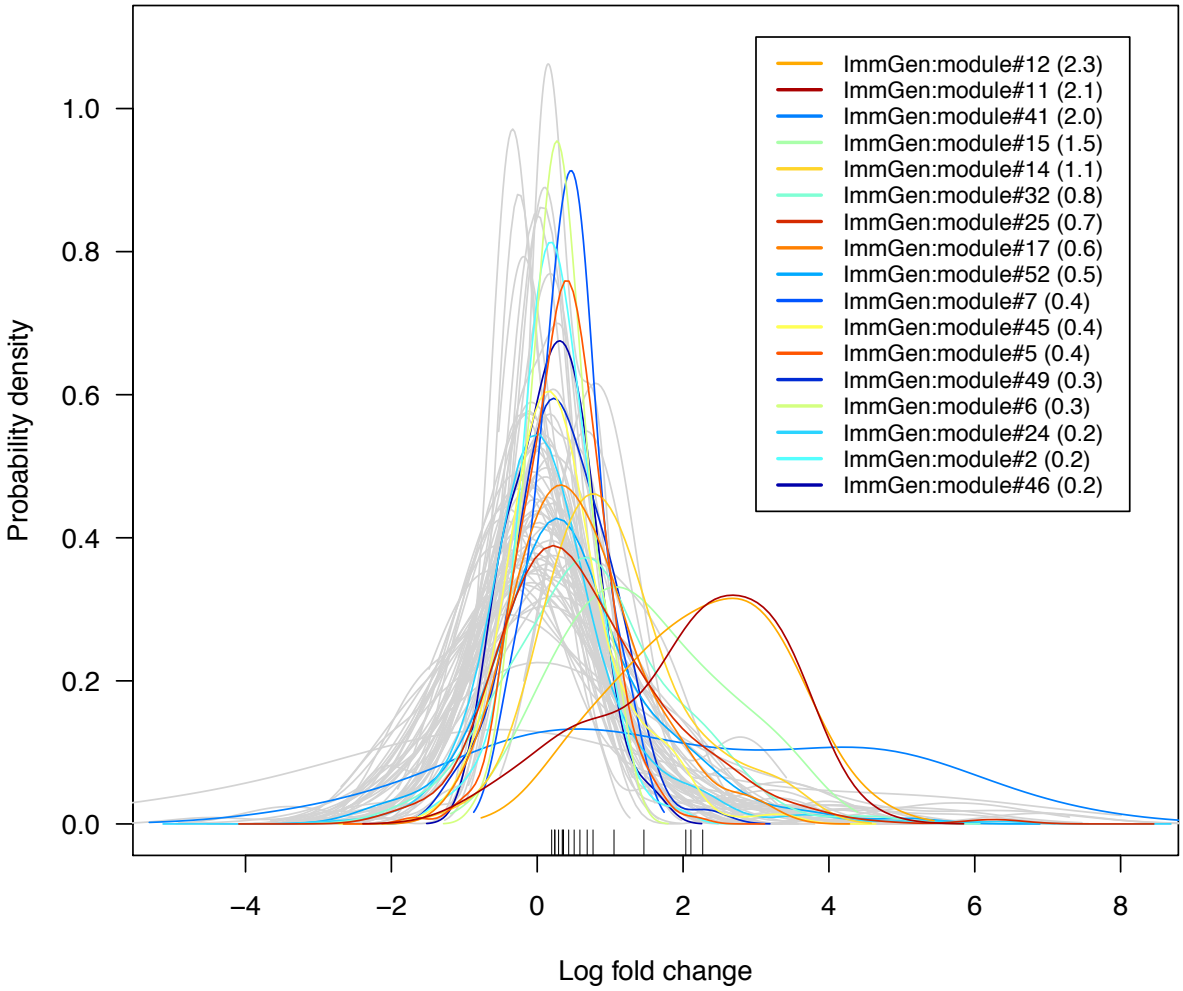
Supplementary Information

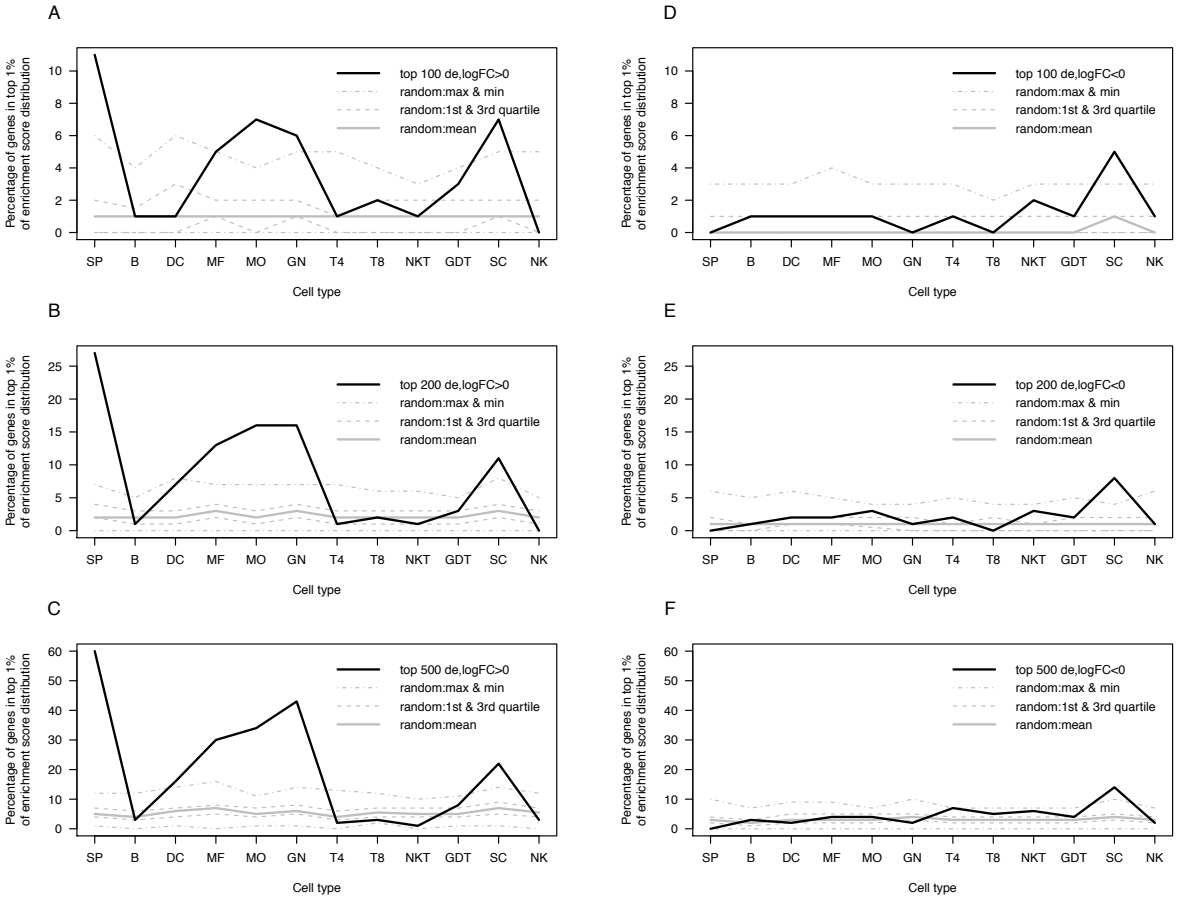


Supplementary Figure 1 Summary of transcriptome analysis between catheterized and naïve mice. Female C57BL/6 mice were implanted with a 5 mm silicon catheter for 24 hours. Naïve control animals did not receive a catheter. Animals were sacrificed and bladders processed for RNA recovery. Experiment was performed 2 times, n=3 mice/experimental group. Differential expression statistics were calculated between catheterized and naïve groups of animals. **(A)** The histogram depicts raw *P*-values from differential expression statistics for 14226 genes. **(B)** The mean average plot shows the mean normalized read count on a $\log_2$ scale on the

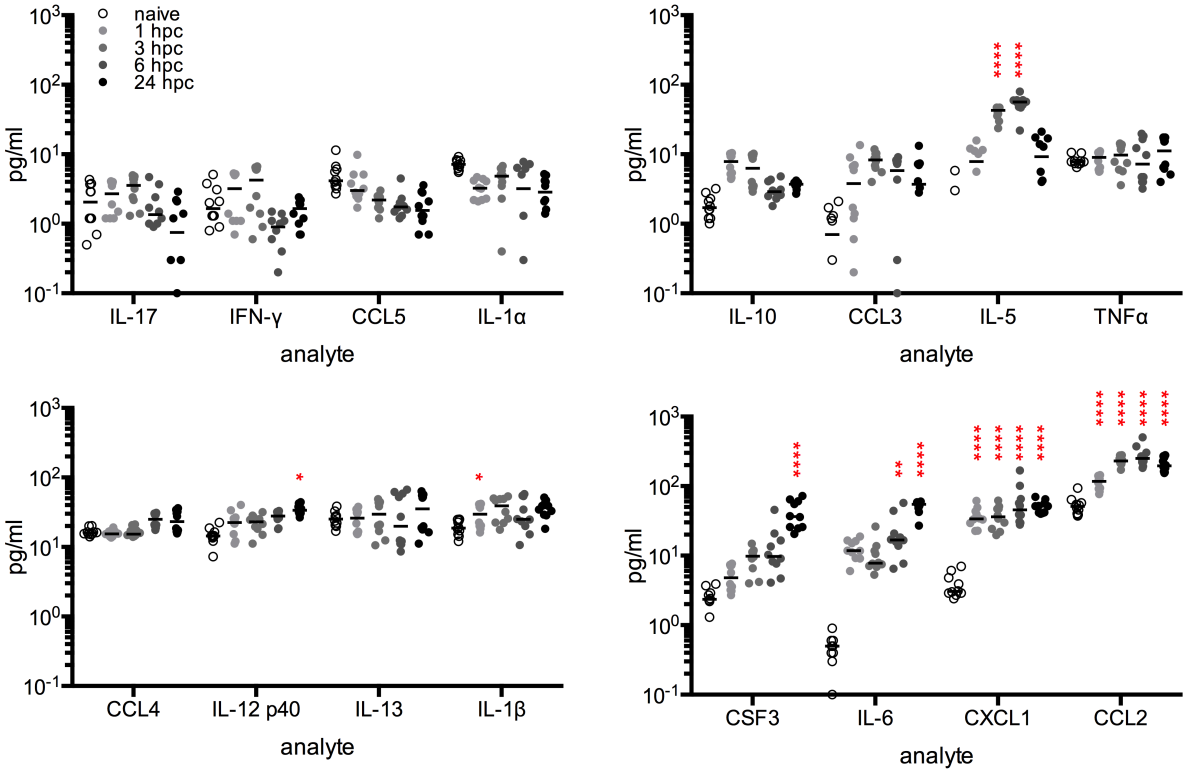
x-axis and the *y*-axis is $\log_2$ ratio between catheterized and naïve animals. **(C**) The volcano plot shows the $\log_2$ expression ratio between catheterized and naïve animals on the *x*-axis and the *y*-axis shows the adjusted *P*-values for each gene. **(D)** The mean-vs-significance plot depicts mean normalized read count on a $\log_2$ scale on the *x*-axis and the *y*-axis shows the adjusted *P*-values for each gene. In panels **(B-D)**, black data points denote the top 100 differentially expressed (d.e.) genes (as defined by ranked adjusted *P*-value), blue points denote the top 200 d.e. genes (excluding those in the top 100) and red points denote the top 500 d.e. genes (excluding those in the top 200). Dark grey data points are all genes showing an adjusted P-value <0.05. The remaining light grey points have adjusted *P*-values > 0.05.



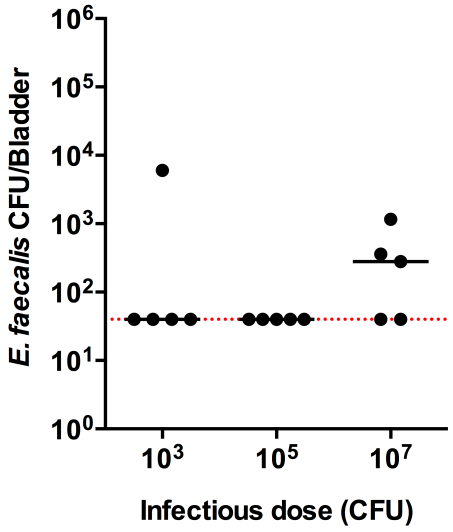
Supplementary Figure 2 Statistical analysis of gene expression changes between catheterized and naïve mice using ImmGen coarse module classification. The stacked density plot shows the distribution of log fold change between catheterized and non-catheterized animals in each of the ImmGen coarse modules that had at least 3 genes detected (79 of 81 modules; see **Table S3** for module annotations and analysis summary). Modules that demonstrated statistically significant differences in their mean $\log_2$ fold change value are highlighted in color and indicated in the inset key. Numbers in parentheses are the mean $\log_2$ fold change.



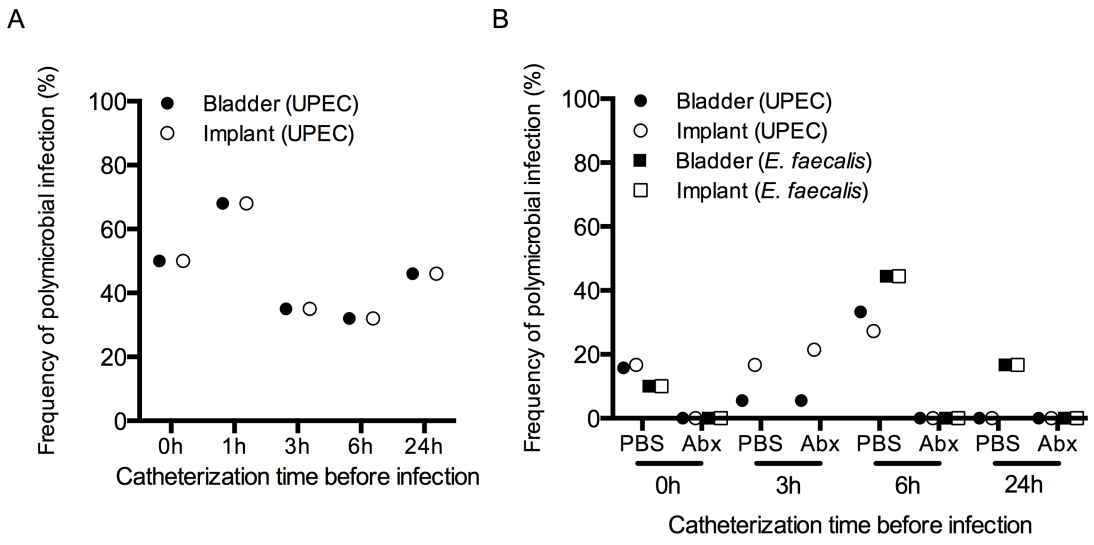
Supplementary Figure 3 Analysis of cell-type enrichment score in sets of top ranked significantly differentially expressed genes that are upregulated or down-regulated in catheterized *vs* naïve animals. The enrichment score of Benita *et al.* (51) was calculated for each cell type from the Immgen data and the genes up-regulated in the top **(A)** 100, **(B)** 200, and **(C)** 500 ranked genes and down-regulated in the top **(D)** 100, **(E)** 200, and **(F)** 500 ranked genes. To identify genes in our analysis that were associated with specificity or selectivity for a given cell type, we calculated the percentage of those genes that were contained in the top 1% of the cell-type specific enrichment score distribution (solid black line compared against 100 sets of randomly selected genes from a matched mean expression range (the random distribution is summarized within each cell type by minimum, 25th percentile, mean, 75th percentile and maximum values; grey lines).



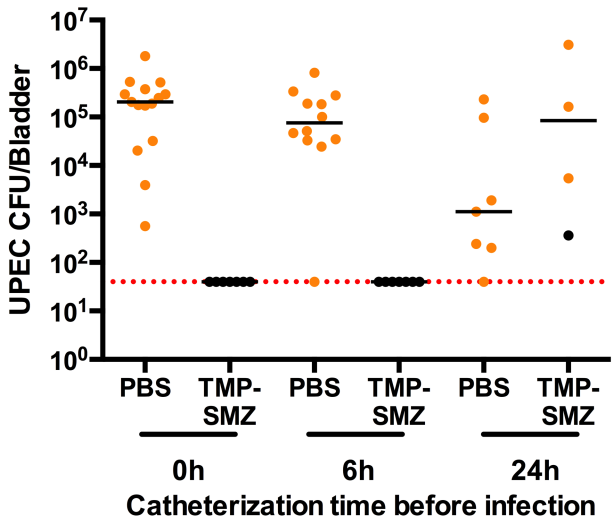
Supplementary Figure 4 Catheterization induces cytokine expression. Naïve female C57BL/6 mice were implanted with catheters. At the indicated time post catheterization, bladders were recovered and homogenized, clarified, and supernatants were analyzed by Luminex technology to determine absolute levels (pg/ml) of the indicated cytokines. The experiment was performed 2 times with n=5 mice per experimental group and pooled. Legend for all 4 graphs is depicted in top left graph. Data represent the mean ± SD. * = $p < 0.05$, ** = $p < 0.01$, **** = $p < 0.0001$ for comparison of catheterized mice to the naïve control group, 2-way ANOVA with Dunnett's multiple comparisons test.



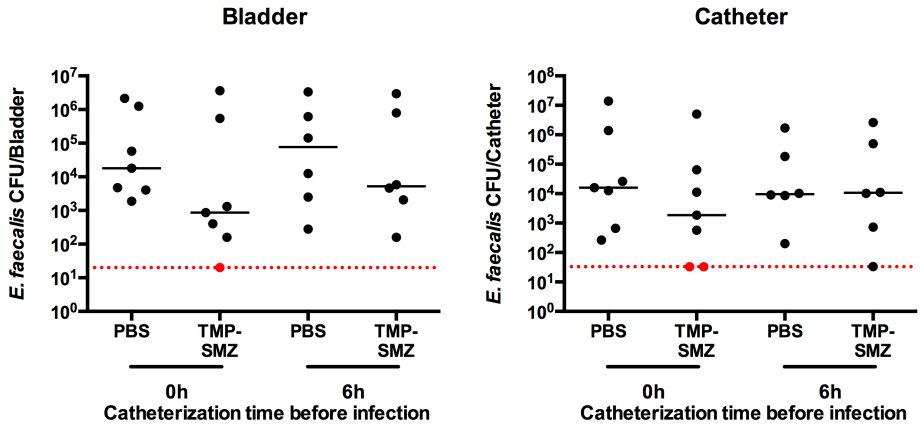
Supplementary Figure 5 The *E. faecalis* ID_{90} dose for catheterized animals does not result in colonization in non-catheterized animals. Naïve C57BL/6 female mice were infected with the indicated colony forming units (CFU) of *E. faecalis*. 24 hours later bacterial burden in the bladder was assessed. Each dot represents one mouse. Experiment was performed twice, n=5 mice per experimental group. Representative data from one experiment are shown.



Supplementary Figure 6 Polymicrobial species can be detected in catheterized mice. (**A-B**) Naïve C57BL/6 female mice were implanted with catheters. At the indicated time post catheterization, mice were infected with ~10^4 colony forming units (CFU) of UPEC or *E. faecalis*. The graphs depict the overall frequency with which bacteria other than UPEC or *E. faecalis* were detected in all pooled data shown in (**A**) **Figure 3 (**n=5-10 mice per experimental group, experiment performed 3-5 times) and (**B**) **Figure 4 (**n=7 mice per experimental group, experiment performed 3 times). 16S rRNA sequencing was used to identify a subset of bacterial strains arising from these infections, which included *Staphylococcus lentus,* and *Staphylococcus xylosus* or *Staphylococcus saprophyticus.* Note that no polymicrobial infection was detected in animals infected with *E. faecalis* from experiments depicted in **Figure 3**.



Supplementary Figure 7 Bacteria are not detected in the urine of antibiotic-treated mice in the absence of bladder infection. Trimethoprim/sulfamethoxazole (TMP-SMZ) or PBS were administered intraperitoneally to naïve female mice concurrent with catheter implantation. Cohorts of animals were then infected with approximately $1x10^4$ colony forming units (CFU) of UPEC at the same time (**0h**) or at the indicated timepoint post catheterization (**6h, 24h**). Graph indicates CFU per bladder assessed 24 hours following infection. Bacterial presence or absence in urine was determined by plating urine samples collected just prior to sacrifice. Mice with positive urine cultures are indicated in orange, mice with negative urine cultures are indicated in black. Each dot is one mouse, the experiment was performed 2 times, n=7 mice per experimental group, and the data pooled. Red dashed line indicates the limit of detection for the assay. The presence of the catheter was verified at the time of sacrifice and mice without a catheter were excluded from the analysis.



Supplementary Figure 8 *E. faecalis* strain OG1RF is resistant to TMP-SMZ.

Trimethoprim/sulfamethoxazole (TMP-SMZ) or PBS were administered intraperitoneally to naïve female mice concurrent with catheter implantation. Cohorts of animals were then infected with $1x10^4$ colony forming units (CFU) of *E. faecalis* at the same time or at the indicated timepoints post catheterization and CFU per bladder and catheter were assessed 24 hours following infection. Each dot is one mouse, red dots indicate animals that were negative for *E. faecalis*, but positive for an unknown bacterial species. The experiment was performed 1 time, n=7 mice per experimental group. The presence of the catheter was verified at the time of sacrifice and mice without a catheter were excluded from the analysis.