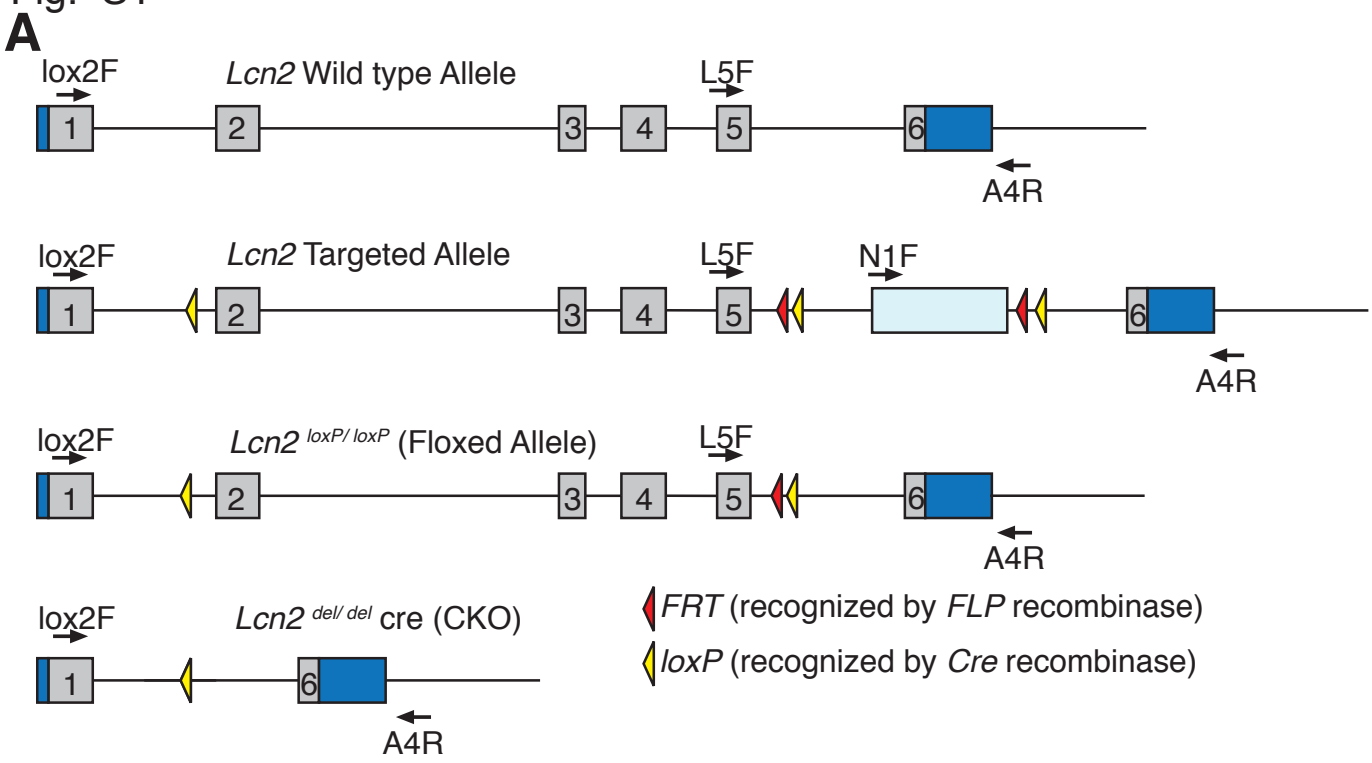
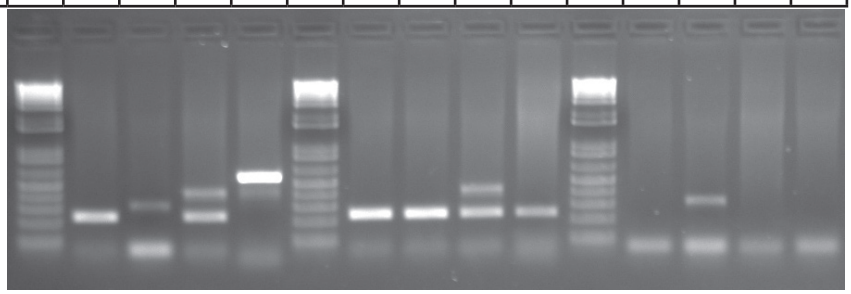
Supplemental Figure S1: **Generation of the *LCN2*$^{loxP/loxP}$ mice.** (**A**) A *loxP* site was inserted into intron 1 (yellow arrowhead), and a neomycin cassette (light blue box) was inserted into intron 5 flanked by *FRT* (red arrowhead) and *loxP* sites. The neomycin cassette was excised by crossing *LCN2*$^{loxPneo/+}$ with FLP Recombinase (FLP) mice generating *LCN2* $^{loxp/+}$. *LCN2*$^{loxp/+}$ was bred against C57BL/6 animals to remove FLP and then subsequently bred with EIIa-*Cre* mice to induce recombination between the intron1 *loxP* and the intron5 *loxP* creating *LCN2*$^{del/+}$, EIIa-*Cre* mice. (**B**) Genotyping strategy. Note that only the wild type allele (250 bp) was amplified with L5F and A4R primers because the PCR parameters did not amplify across Neomycin cassette in the "targeted allele". (**C**) Recombination in the Cre-deleted allele was authenticated by sequencing the Lox2F-A4R PCR product.

Fig. S1



B

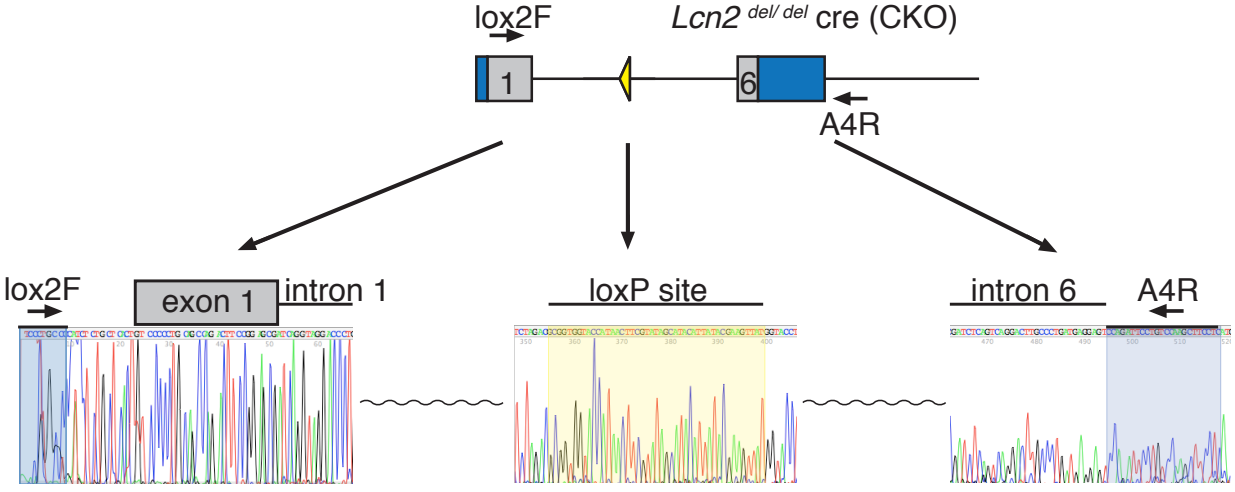
Wild type		X					X					X			
Targeted Allele			X					X					X		
Floxed Allele				X					X					X	
Cre-deleted Allele					X					X					X
1 Kb Plus Ladder	X					X					X				



Primers Pairs

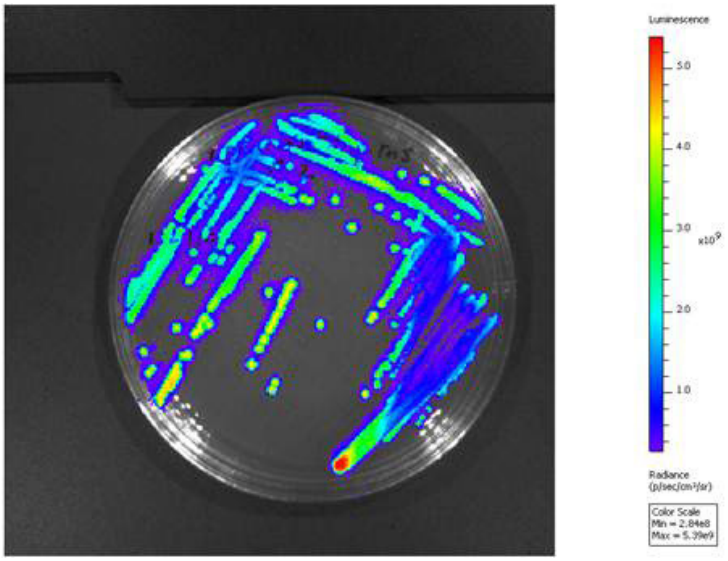
L5F / A4R | N1F / A4R | L5F / A4R | lox2F / A4R | L5F / A4R | N1F / A4R

C



Supplemental Figure S2: **UPEC-Lux.** Lux operon was inserted by conjugation at the Tn site with the Tn5-lux system in Gram-negative (CFT073) bacteria. Positive conjugates were selected on LB agar plates for luminescent signal.

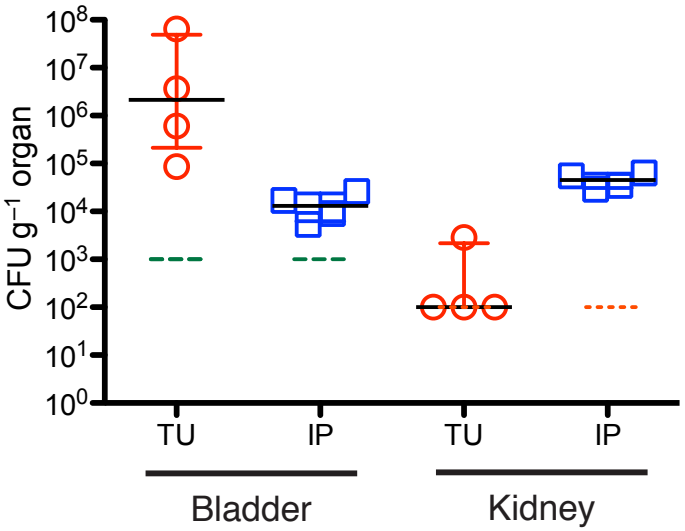
Fig. S2


Luminescence
5.0
4.0
3.0
x10^9
2.0
1.0
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 2.84e8
Max = 5.39e9

Supplemental Figure S3: Analysis of kidney and bladder in C57BL/6 infected mice (cystitis model) (**A**) Recovery of UPEC from kidney and bladder 1d post-transurethral inoculation (TU). Intraperitoneal (IP) inoculation served as a positive control (*n* = 4 each). CFUs in the kidney of the TU inoculated mice were at/below the limit of detection (kidney LOD = 10^2, red hashed line; bladder LOD = 10^3 green dotted line).

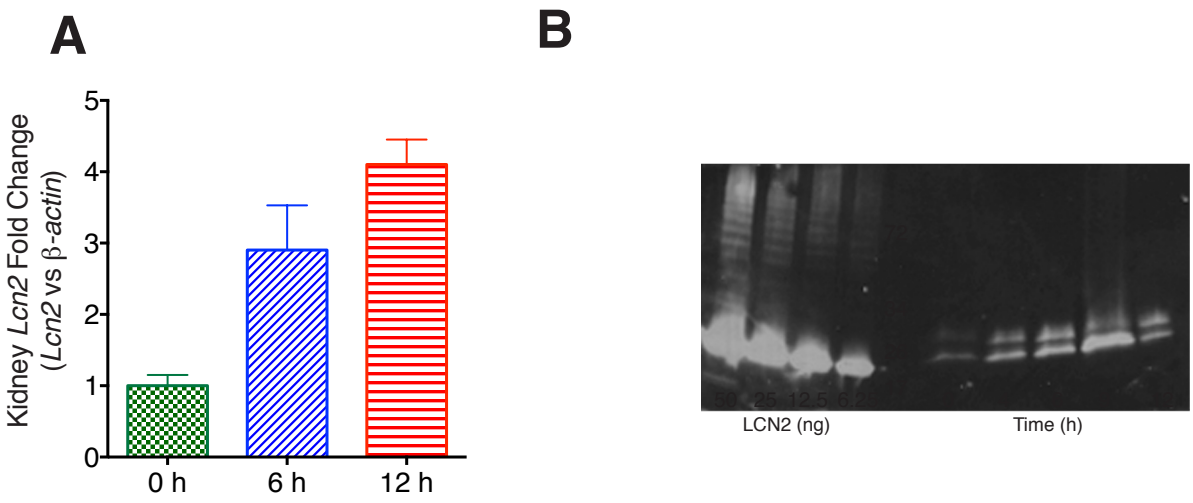
Fig. S3

A


10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
CFU g^{-1} organ
TU
IP
TU
IP
Bladder
Kidney

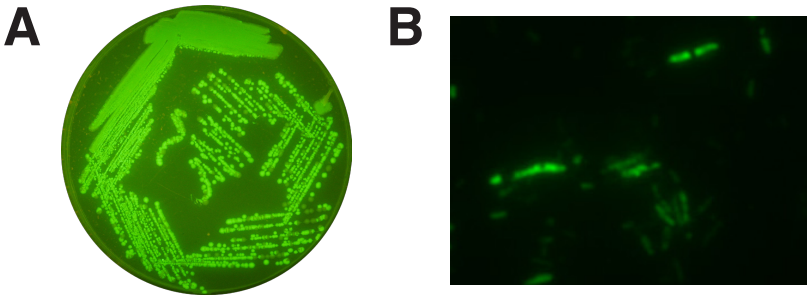
Supplemental Figure S4: Non-canonical LCN2 Activation: (**A**) We found on average a 2.9 ± 0.68 fold increase in *Lcn2* message ($n = 5$, $P = 0.0369$) at 6 h post Ent treatment and a 4.1 ± 0.35 ($n = 2$, $P = 0.0382$) fold increase at 12 h. (**B**) uLCN2 was detected in mouse urine after *i.p.* injection of Ent (peak 9 h).

Fig. S4


A
B
Kidney Lcn2 Fold Change
(Lcn2 vs β-actin)
5
4
3
2
1
0
0 h
6 h
12 h
50 25 12.5 6
LCN2 (ng)
Time (h)

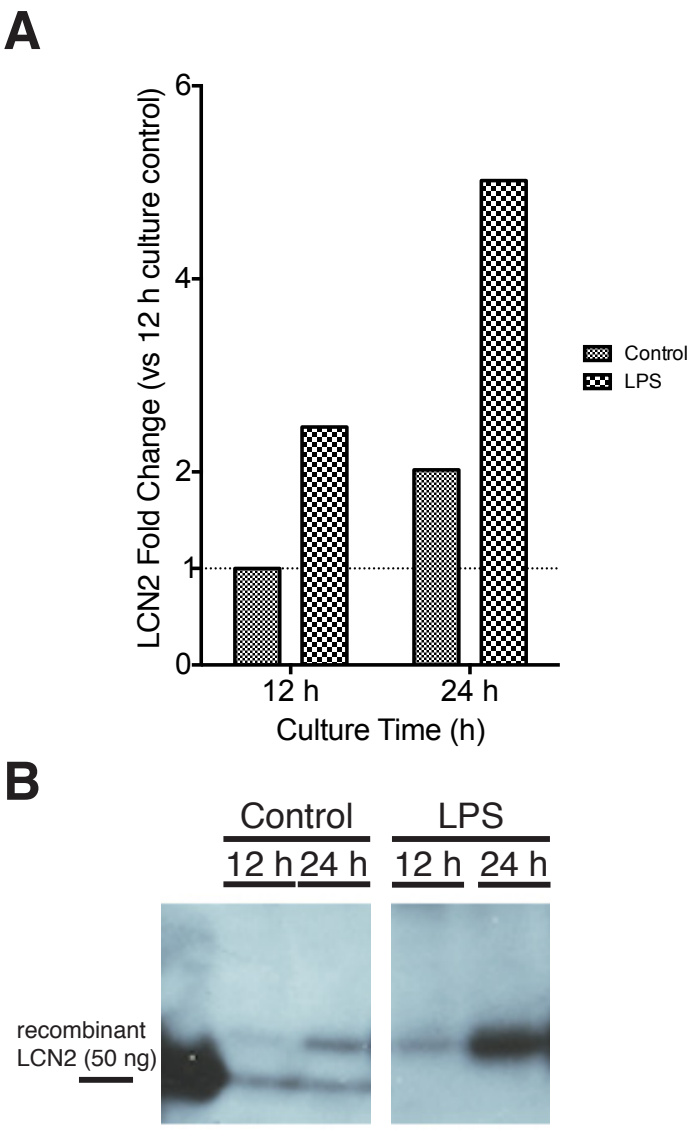
Supplemental Figure S5: **UPEC-GFP.** GFPuv was expressed under control of the *E.coli lacZ* promoter (pGFPuv plasmid, Clontech). (**A**) UPEC-GFP visible on LB agar plates. (**B**) Rod shaped UPEC-GFP were seen at high power (100x).

Fig. S5



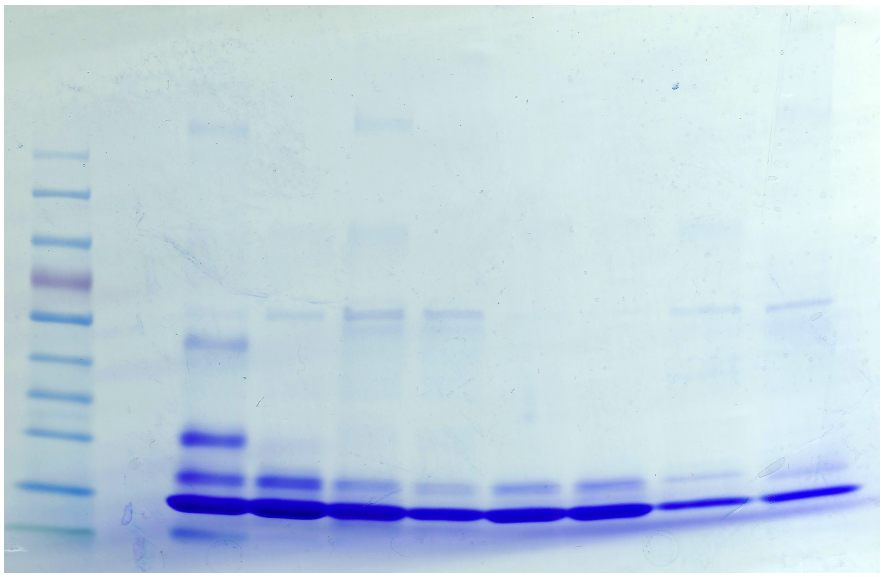
Supplemental Figure S6: **Secretion of LCN2 from bladder**. (**A**) C57BL/6 mice were treated with LPS (*i.p.* 1 mg kg^{-1}) and bladders explanted on a filter. LCN2 was detected in the culture media. Bladders from LPS treated mice secreted 2 fold (12 h of explant) and 5 fold (24 h of explant) higher levels of LCN2 than did control bladders (n = 3 independent experiments). (**B**) LCN2 was detected by western blot using 100% of media conditioned by 6 bladders. Comparison with standard protein (50 ng), demonstrated that explanted bladders secreted LCN2.

Fig. S6


A
LCN2 Fold Change (vs 12 h culture control)
6
4
2
1
0
12 h
24 h
Culture Time (h)
Control
LPS
B
Control
LPS
12 h
24 h
12 h
24 h
recombinant
LCN2 (50 ng)

Supplemental Figure S7: **Coomassie Brilliant Blue stain of WT and KO urine.** *Tcfcp2l1* $^{flox/flox}$; Ksp-Cre (IC-Knockout) animals did not show excess proteinuria.

Fig. S7



Supplemental Table 1: List of PCR and qPCR primers

Table S1

Lcn2 flox/flox genotyping primers

Primer Name	
lox2F	5' - AAGACTCAACTCAGAACTTGATCC - 3'
A4R	5' - GAGGAAGCTTGGACAGGAATCTGG - 3'
L5F	5' - ACGACAACATCATCTTCTCTGTCC - 3'
N1F	5' - TGCGAGGCCAGAGGCCACTTGTGTAGC - 3'
Lcn2R	5' - GTCCTTCTCACTTTGACAGAAGTCAGG - 3'
Lcn2F	5' - CACATCTCATGCTGCTCAGATAGCCAC - 3'

QPCR primers

Standard QPCR

Gene Symbol	
Lcn2	
Forward	5' - CTCAGAACTTGATCCCTGCC - 3'
Reverse	5' - TCCTTGAGGCCCAGAGACTT - 3'
bact	
Forward	5' - CTAAGGCCAACCGTGAAAAG - 3'
Reverse	5' - TCTCAGCTGTGGTGGTGAAG - 3'
gapA	
Forward	5' - AAGTTGGTGTTGACGTTGTCGC - 3'
Reverse	5' - AGCGCCTTTAACGAACATCG - 3'
chuA	
Forward	5' - AGCGTGTTGAGATTGTTCGC - 3'
Reverse	5' - AAACCACTGCTTTGTCCTTCCTGC - 3'
chuS	
Forward	5' - CTGTTTCTCAATCAATGGGCCAGTG - 3'
Reverse	5' - TATGCCACCGACAATACCGATATGG - 3'
chuT	
Forward	5' - AGCTGGACTTTTAGCGTAACGGCTG - 3'
Reverse	5' - CGACATCTTATCCACCAGAAACCGC - 3'
chuW	
Forward	5' - GCCACTGGCCCCTGATTGTGAA - 3'
Reverse	5' - AATCCGCAAGAAAATGGCCCG - 3'
entA	
Forward	5' - TAGCTGAAACGGAGCGACTGGACG - 3'
Reverse	5' - ACGTCGGCGGTGCGTTTAACCT - 3'
entC	
Forward	5' - CCAAAGCGCAGGGCATCAAA - 3'
Reverse	5' - AAAACAAGCTTCCGCACGCCG - 3'
entE	
Forward	5' - AGCGGGGATGATTTCCTCAACAC - 3'
Reverse	5' - GCTGAGGATTTTACTGCCACGCC - 3'
entF	
Forward	5' - CGCTGTTCGGTCCGGTACTCAA - 3'
Reverse	5' - TGAACTGGCCCTGTTCCCGGAT - 3'
fhuA	
Forward	5' - ACGGCCAAAGCCAGAATAAC - 3'
Reverse	5' - TTACCGTAAAGCACGGAAACCG - 3'
iroB	
Forward	5' - CCGGTCTGGATTCCGAAGCTGGTTA - 3'
Reverse	AGACCATCTGGTGGAGTTTGCCG - 3'
iroN	
Forward	5' - ATTACCAAACGTCCCACCAACG - 3'
Reverse	5' - AAACGCGTGGTAAGAGCATCAC - 3'
iucA	
Forward	5' - CCCATACGCAACAGGCAATTGATG - 3'
Reverse	5' - CACCCTGCGCCTAAGTCTCATGA - 3'
iucD	
Forward	5' - CTTCAGCGAAGAAATGGCAGACCA - 3'
Reverse	5' - TGATATACCGGTCGTGATGCAAACC - 3'
sitB	
Forward	5' - GGCGTTACAGAAAAACCTGGTTGCC - 3'
Reverse	5' - ATGGGTATGTTGCGTATCGCCAAA - 3'
sitA	
Forward	5' - GCACTCACCTGCTCGATCGCAT - 3'
Reverse	5' - TCTCATCCATAACCAAGCCTGGTGC - 3'

Taqman Genes

Gene Symbol	Assay ID
Actb	Mm01205647_g1
Ccl2	Mm00441242_m1
Ccl5	Mm01302427_m1
Csf3	Mm00438334_m1
Cxcl1	Mm01354329_g1
Cxcl10	Mm00445235_m1
Cxcl12	Mm00445552_m1
Il18	Mm00434225_m1
Il1a	Mm00439621_m1
Il1b	Mm00434228_m1
Il2	Mm00434256_m1
Il6	Mm00446191_m1
Jun	Mm00495062_s1
Lcn2	Mm01324470_m1