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Supplemental Information

Natural Killer Cell-Mediated Host Defense against Uropathogenic *E. coli* Is Counteracted by Bacterial Hemolysina-Dependent Killing of NK Cells

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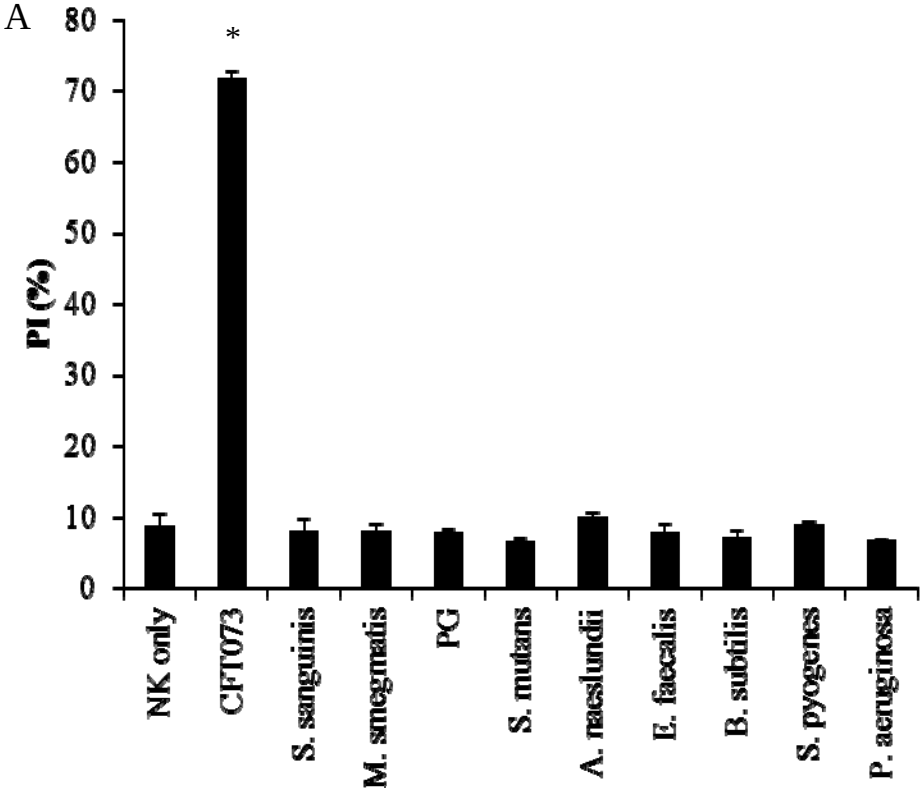
A

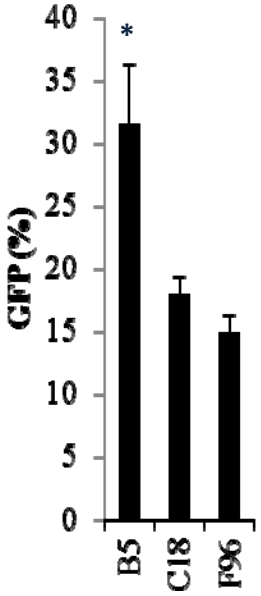


B

bacteria	medium	conditions
P. gingivalis (PG)	wilkins	anaerobic
S. sanguinis	BHI	5% CO_2
S. mutans ATCC 27375	BHI	5% CO_2
E. faecalis	BHI	5% CO_2
P. aeruginosa	LB	aerobic
B. subtilis (PY79)	LB	aerobic
M. smegmatis MC^2155	TSB+0.05% tween 80	aerobic
UPEC CFT073	LB	aerobic
A. naeslundii	wilkins	anaerobic
S. pyogenes	BHI	5% CO_2

Supplementary Figure 1

A



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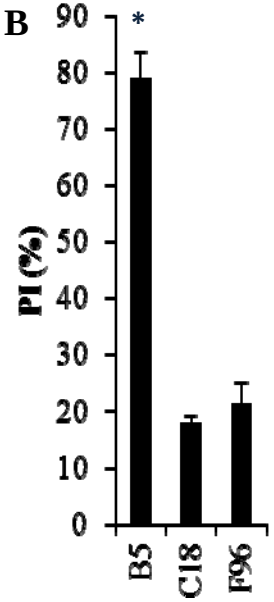
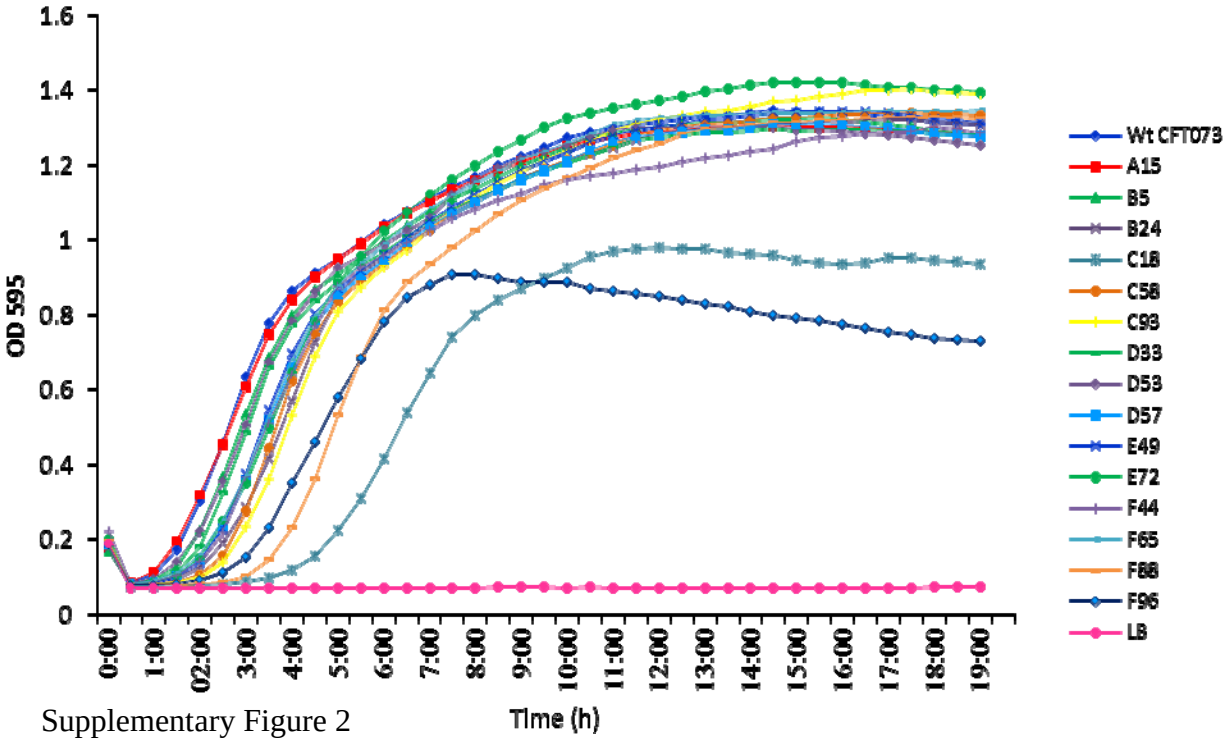


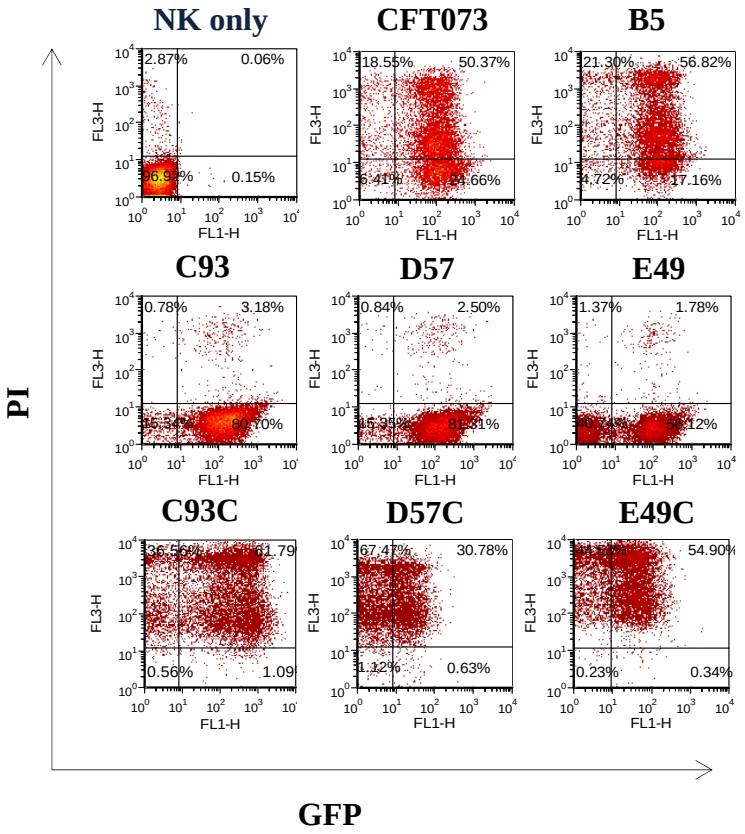
C

Mutant #	Disrupted gene	Putative disruptive function
C18	*GTP-binding protein-lepA*	Elongation factor
F96	*rfaG*	LPS core biosynthesis protein

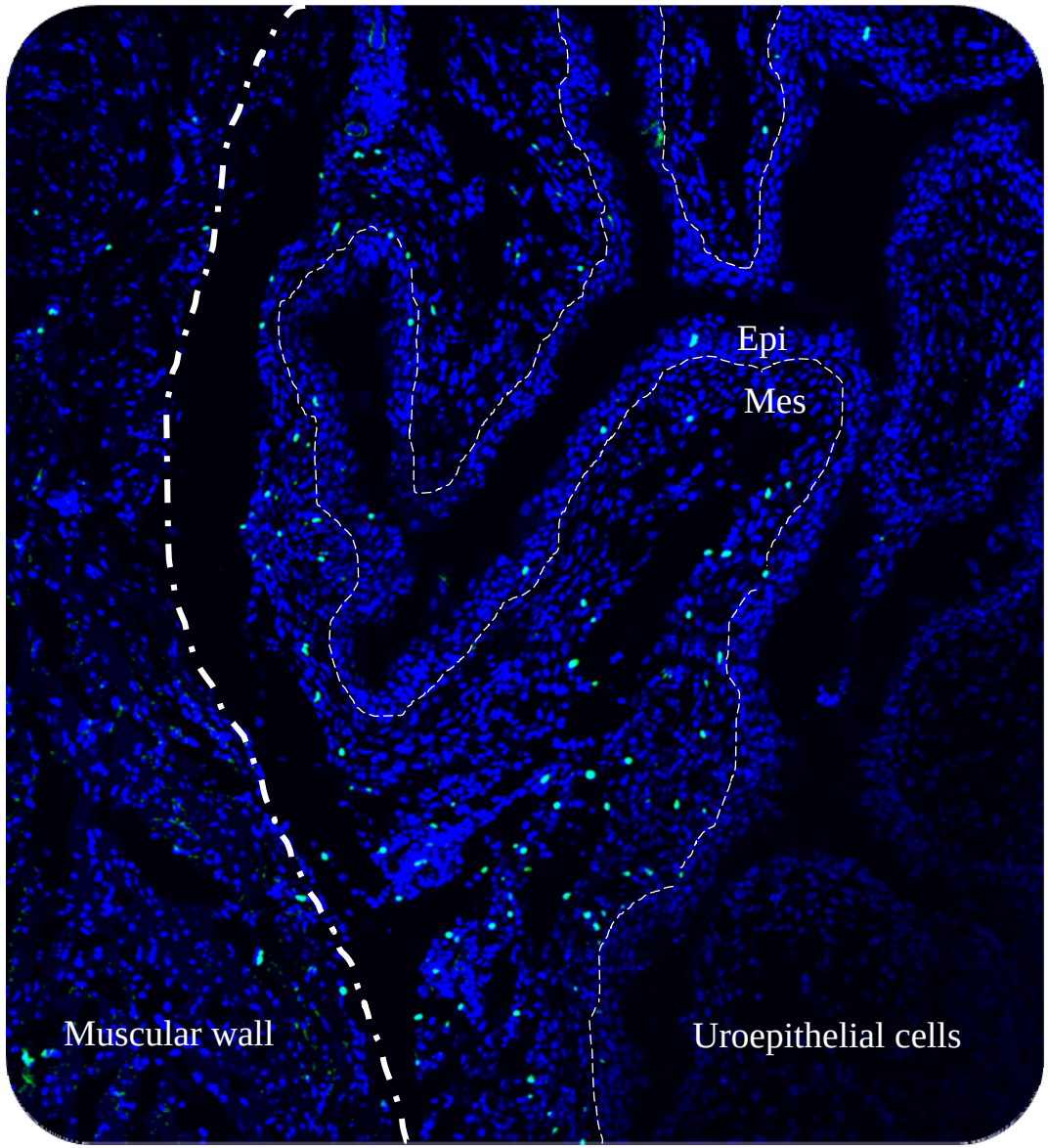
D



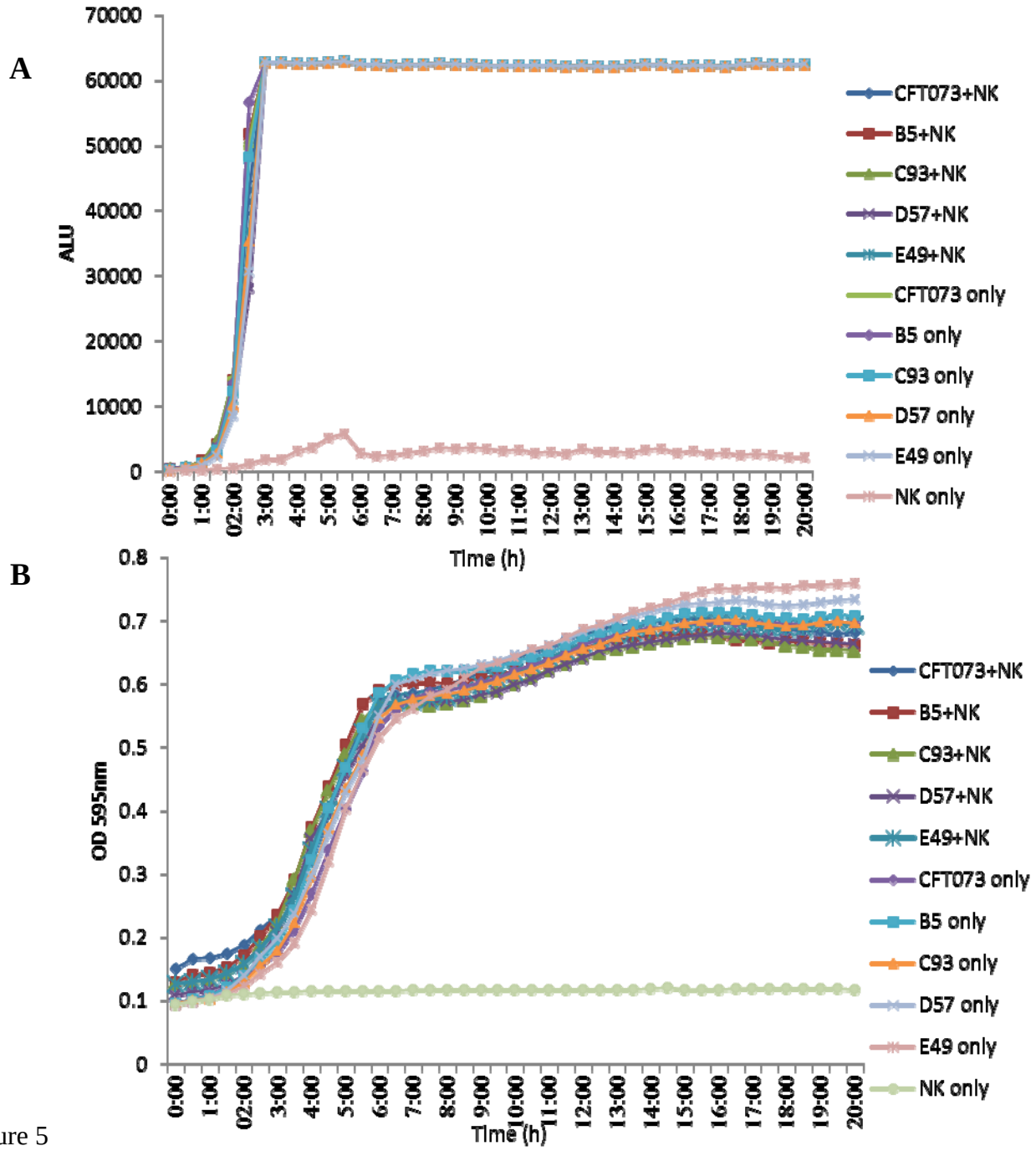
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5

Supplementary figure legends:

Figure S1. Other bacteria used in the NK cell killing experiments, related to Figure 1

(A) Percentages of PI-positive NK cells following 3 hours incubation at 37°C with the various bacteria strains indicated in the x axis. Data are a representative of two independent experiments and an average±SD of duplicate is shown. (B) A table summarizing the various bacteria used in this study and their characterization. The name of the bacteria, their growth medium (medium) and their growth conditions (conditions) are indicated. *P<0.005 for UPEC CFT073 versus all other bacteria.

Figure S2. Two UPEC CFT073 mutants defective in adhesion and in killing, related to Figure 3

(A) Adhesion and killing (B) of GFP-expressing UPEC CFT073 mutants C18, F96. Adhesion was performed at 4°C (A, determined by percentages of GFP positive NK cells) and killing at 37°C (B, determined by PI positive NK cells) respectively. Data are representative of at least three independent experiments. *P<0.001. (C) Table summarizing the genes disrupted in C18 and in F96. (D) Growth curves of the various adhesion and killing mutants investigated in this study.

Figure S3. Killing of NK cells by various *E. coli* strains, related to Figure 5

Representative flow cytometry staining of the various GFP expressing bacteria: the irrelevant mutant B5, the wild type UPEC CFT073 bacterium, the hemolysinA mutants (C93, D57 and E49) and the complemented bacteria (C93C, D57C and E49C). Killing of human NK cells at 37°C is represented by PI staining.

Figure S4. Panoramic view of NK cells accumulation in the bladder, related to Figure 6

Transversal panoramic view of bladder infected with the hemolysinA mutant C93 using confocal microscopy. NK cells are marked with GFP. The uroepithelial cells and the muscular wall are indicated and are separated by a dashed dotted line. Dashed lines indicate the epithelial (epi)-mesenchymal (mes) boundary. Representative panoramic view of six hemolysinA C93 mutant bladders harvested at day 2 following UTI infection is shown.

Figure S5. Bacterial viability following incubation with NK cells, related to Figure 7

Bacteria were transformed with the pEM7-lux plasmid harboring the luciferase genes and incubated for 3 hours at 37^{o}C with the NK cells at a ratio of 1:100 bacteria per cells. A suspension of bacteria at the same concentrations, without NK cells was incubated in RPMI 1640 (without antibiotics) as a control. Luminescence (A) and optical density (B) of bacteria were measured. ALU, Arbitrary luminescence units.

Table S1. Some bacteria and plasmids used in this work, related to Figure 1

Various bacteria (A) and plasmids (B) used in this work.

A

Bacterial strains:	Characterization:	Ref:
CFT073	Pyelonephritis isolate, *fim*$^{+}$, *pap*$^{+}$ *hly*$^{+}$ Nal^{r}	(Mobley et al., 1990)
UPEC SR71	Cystitis clinical isolate, Amp^{r}	
EPEC E2348/69 (#1)	EPEC wild type strain (strain O127:H6) clinical isolation from an outbreak in Taunton UK	(Levine et al., 1985; Taylor, 1970)
ATCC 25922	Clinical isolate	
XL-1 blue	Stratagene	
UPEC76 (PAP-)	CFT073 (Nal^{r}) derivative; both copies of P fimbrial operon disrupted	(Mobley et al., 1993)
CFT073-OFF (fim-off)	fim invertible element locked off	(Gunther et al., 2002)
CFT073-ON (fim-on)	fim invertible element locked on	(Gunther et al., 2002)
CFT073 fim-pap-	UPEC76 with a deletion of the type 1 fimbrial operon fimA-H	(Snyder et al., 2005)

B

Plasmids:	Characterization:	Ref:
pMODKan	pMOD-3<R6Kγ*ori*/MCS> based, Source of Kan transposon. Amp^{r}, Kan^{r}	(Bahar et al., 2009)
pGNH404	pUC18 based, CFT073 *hly* operon (*hlyCABD*), Amp^{r}	(Mansson et al., 2007)
pCM18	Erm^{r}; pTRKL2-PCP58-RBSII-gfpmut3*-T3-T4	(Hansen et al., 2001)
p_{em}7-lux	pGEN222 based, synthetic em-7 promoter; constitutive *lux* expression, Amp^{r}	(Lane et al., 2007)

Supplementary eferences:

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