Supplementary materials

Supplemental figures

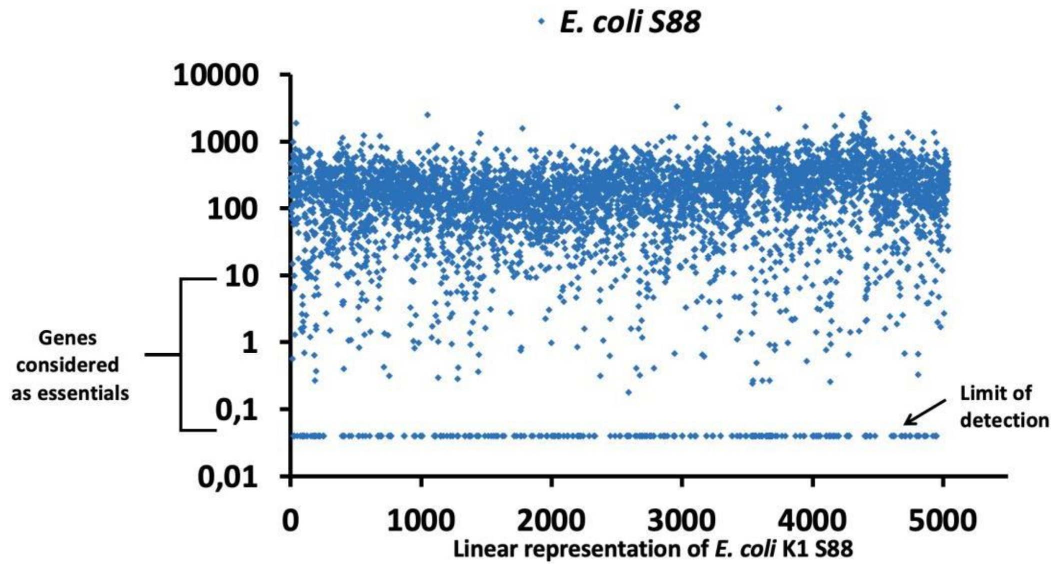


Figure S1. Properties of the *E. coli* K1 S88 TnSeq bank grown in lysogeny broth
Analysis of the overall frequency of Tn insertions into the chromosome was based on use of one million sequencing reads to normalize the data from different DNA preparations (RPKM). In lysogeny broth (LB), the 300·000 *E. coli* K1 S88 Tn-mutants had a relatively homogeneous distribution of Tn insertions across *E. coli* K1 S88 chromosomes with no genes having Tn insertions with more than 10·000 sequencing reads. Following definitions we have previously used, genes with <10 sequencing reads were defined as those essential for growth in LB.

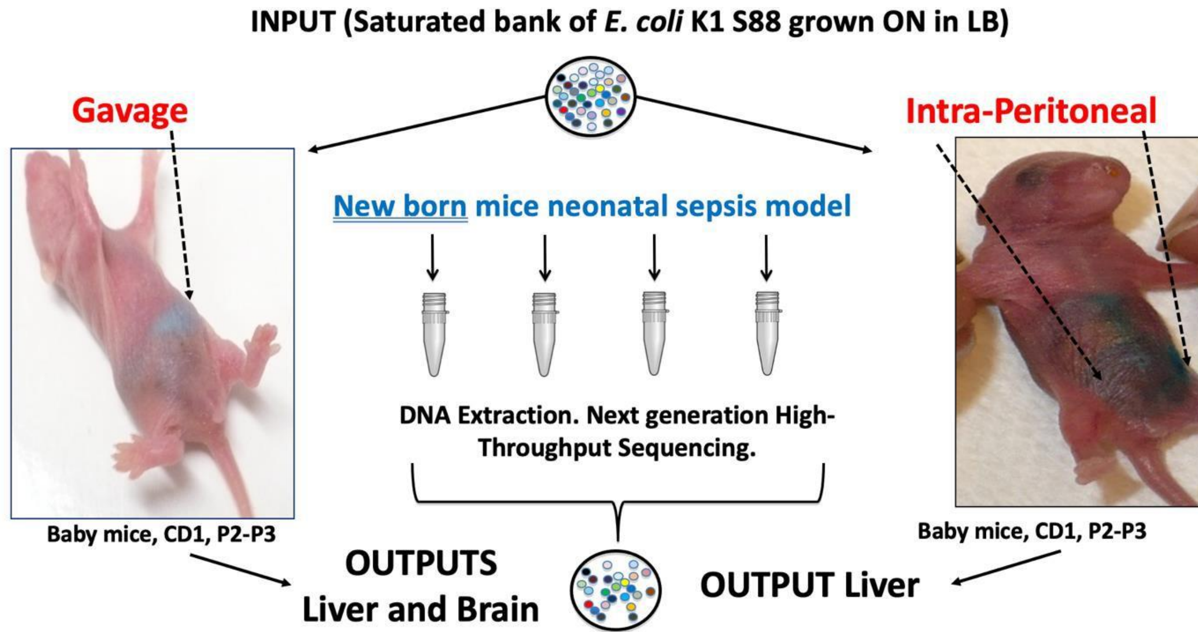


Figure S2. ***E. coli*** **K1, neonatal sepsis model**

The $3x10^5$ saturated bank of *E. coli* K1 S88 grown overnight (ON) in lysogeny broth (LB) was administered by an IP injection or by gavage ($5x10^6$/mouse). Green food dye was used to confirm the intraperitoneal or intragastric location of the bacterial challenge following IP injection or oral gavage of the inoculum. To determine the systemic dissemination in both routes of infection, newborns' livers were harvested 24 hours after the challenge. To study brain infection in the gavage model of neonatal bacterial meningitis, brains were harvested 24 hours after the bacterial challenge. At least $3x\ 10^6$ were recovered from each organ of individual mice. Genomic DNA was extracted, digested by MmeI and gel-sized fragments ligated to oligonucleotides adapters, PCR amplified and sequenced.

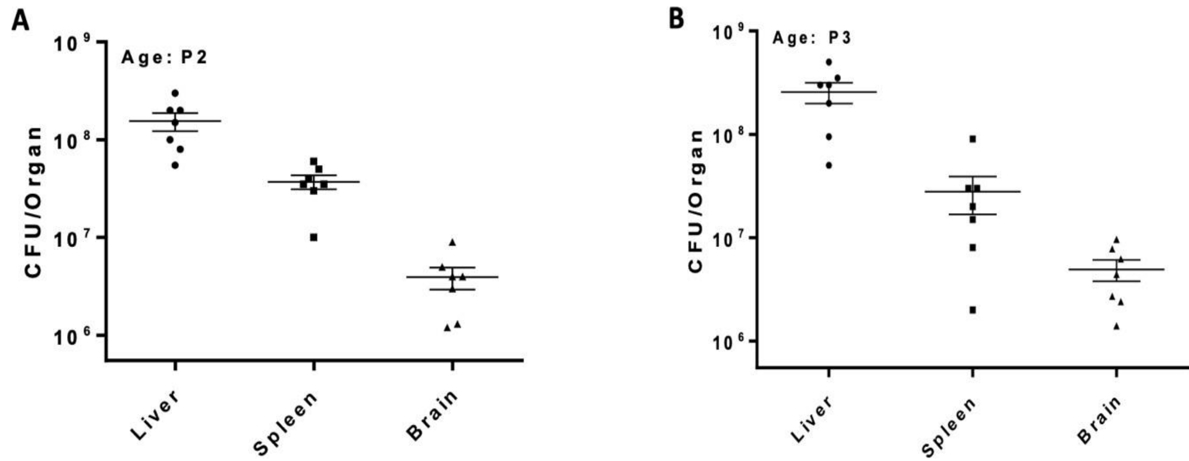


Figure S3. Systemic dissemination of *E. coli* K1 after intraperitoneal infection of new born mice.
Strain: *E. coli* K1 S88. Inoculum: $4x10^6$
Age of the new born mice: 2 days (A) and 3 days (B) old
Bars represent the mean, and error bars depict the 95% confidence interval.

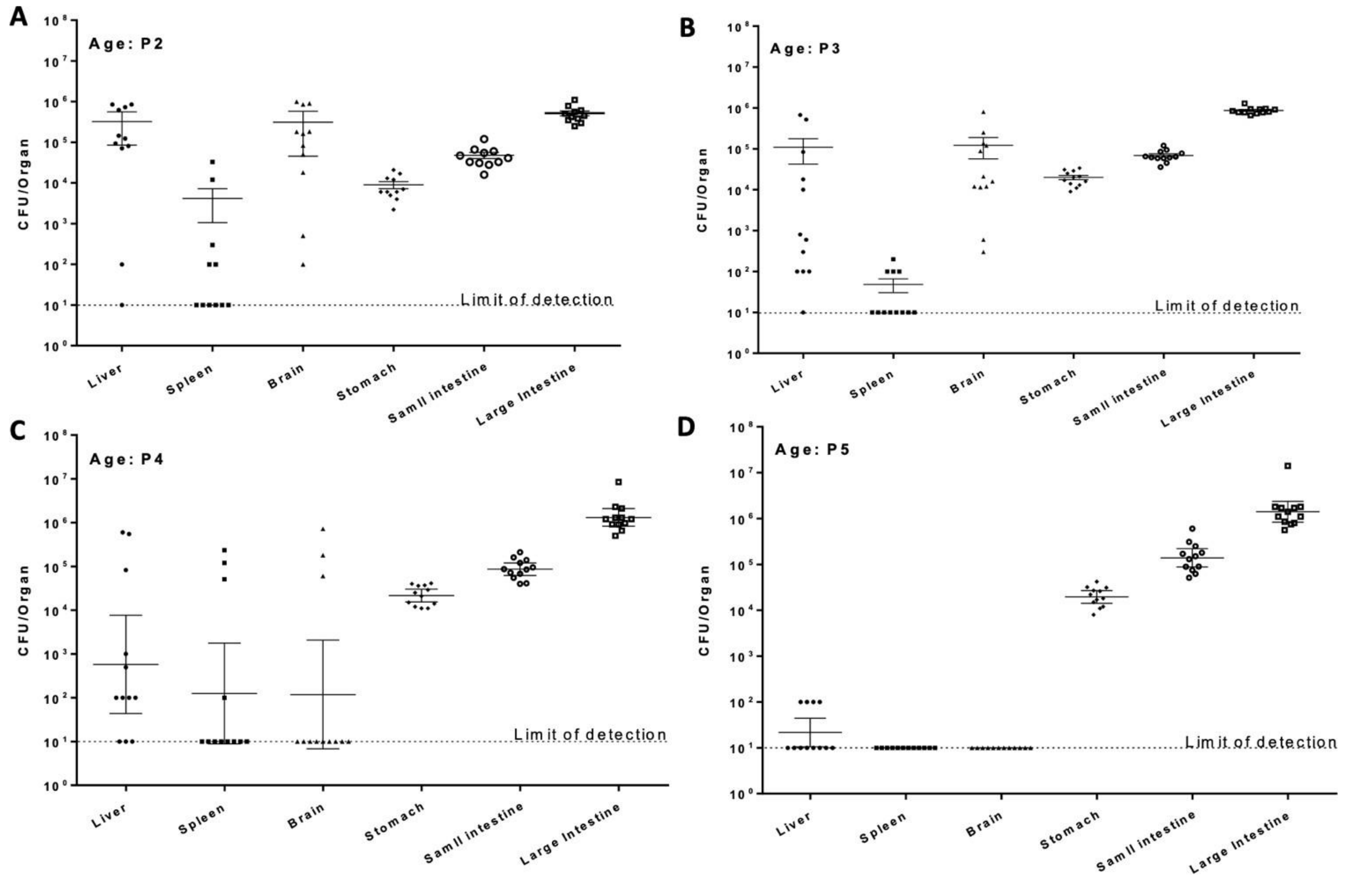


Figure S4. Systemic dissemination of *E. coli* K1 from the gastro-intestinal tract after gavage of new born mice
Strain: *E. coli* K1 S88. Inoculum: $2x10^6$
Age of the neonates: 2 days (A), 3 days (B), 4 days (C) et 5 days old (D)
Bars represent the mean, and error bars depict the 95% confidence interval.

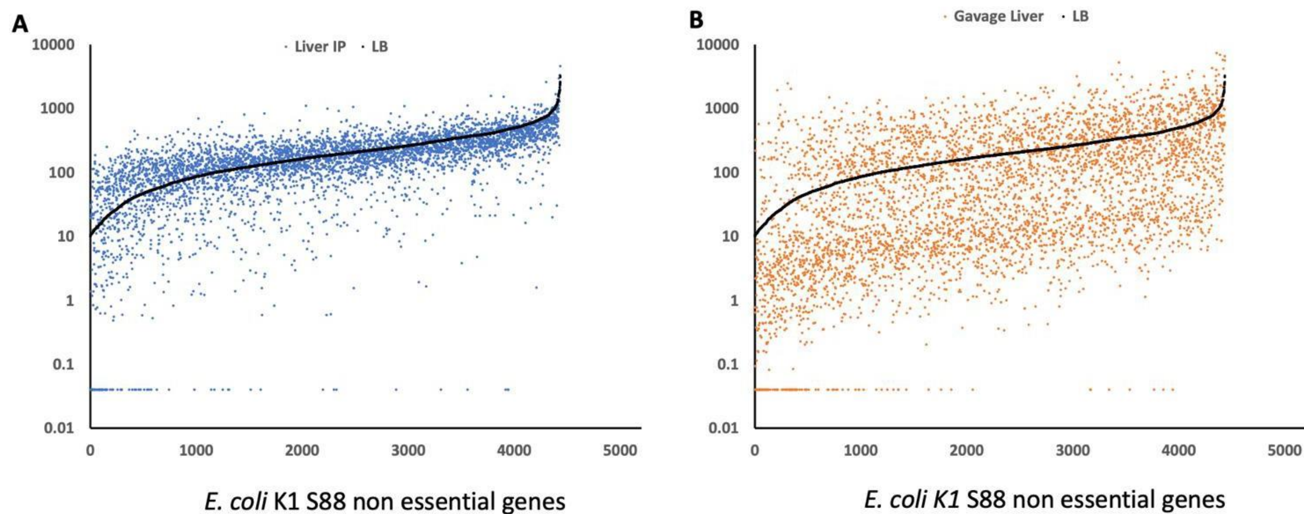


Figure S5. ***In vivo*** **fitness of** ***E. coli*** **K1 S88 Tn library**
Relative ranking and absolute number of RPKM that changed for 4818 non-essential genes of *E. coli* K1 S88 in the new born mice meningitis models, comparing the RPKM in the lysogeny broth (LB) input with those obtained in the liver after IP challenge (A) or gavage (B). Dots above the input lines (in black) indicate Tn insertions in genes with a positive fitness (increased RPKM), whereas dots below the input line indicate those with a negative fitness (decreased RPKM).

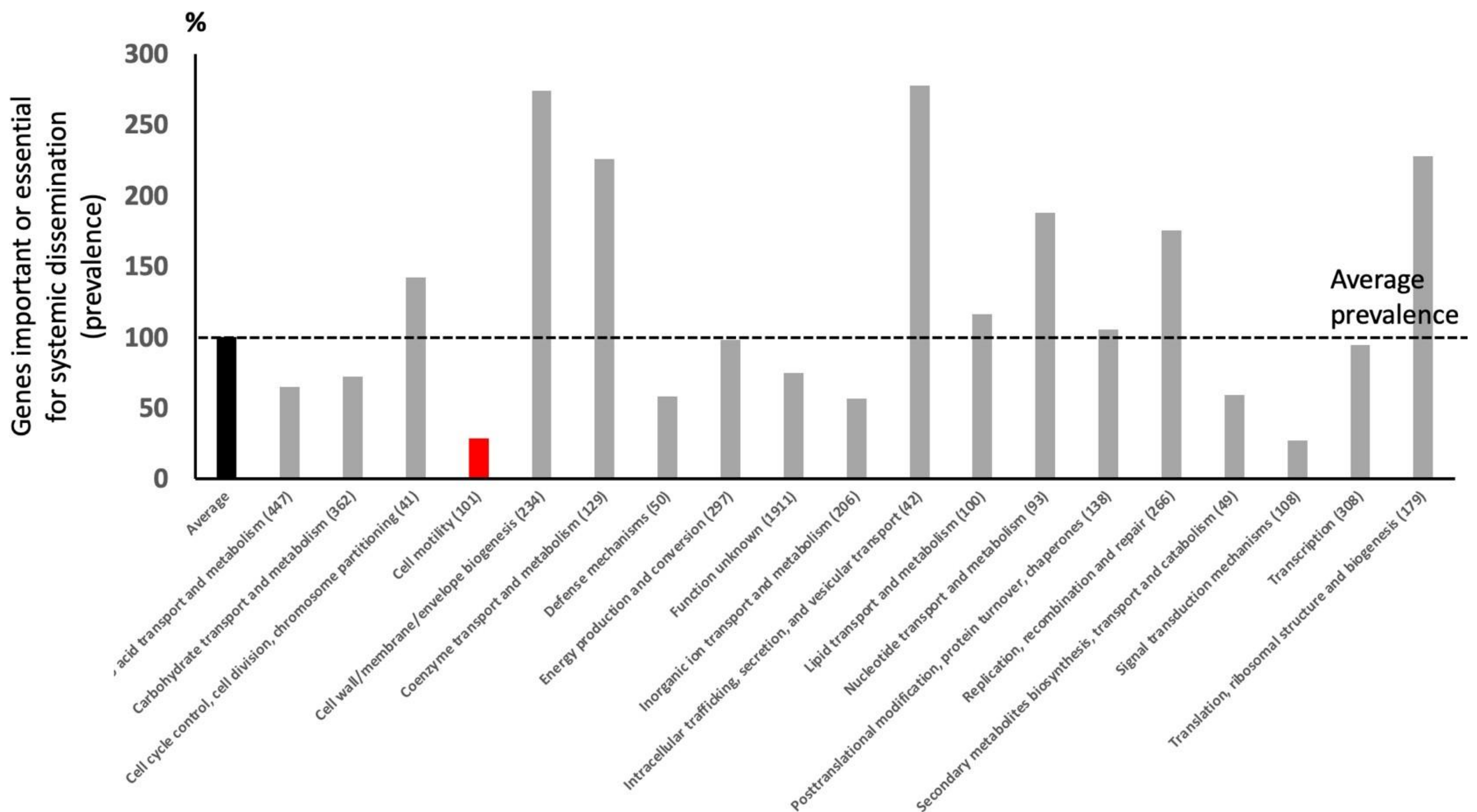


Figure S6. *E. coli K1* important genes for systemic dissemination analyzed by functional classes
E. coli K1 S88 genes were classified into 19 functional classes (https://www.genome.jp/kegg/). The prevalence of genes important or essential for systemic dissemination (defined by more than 10 fold decrease in both routes of infection between the growth in lysogeny broth and the mutants in these genes recovered from the livers) in each functional class is represented, compared to the overall average prevalence of these genes in *E. coli* K1 S88. The red bar represent the importance of the genes belonging to the functional class “motility” in the systemic dissemination.

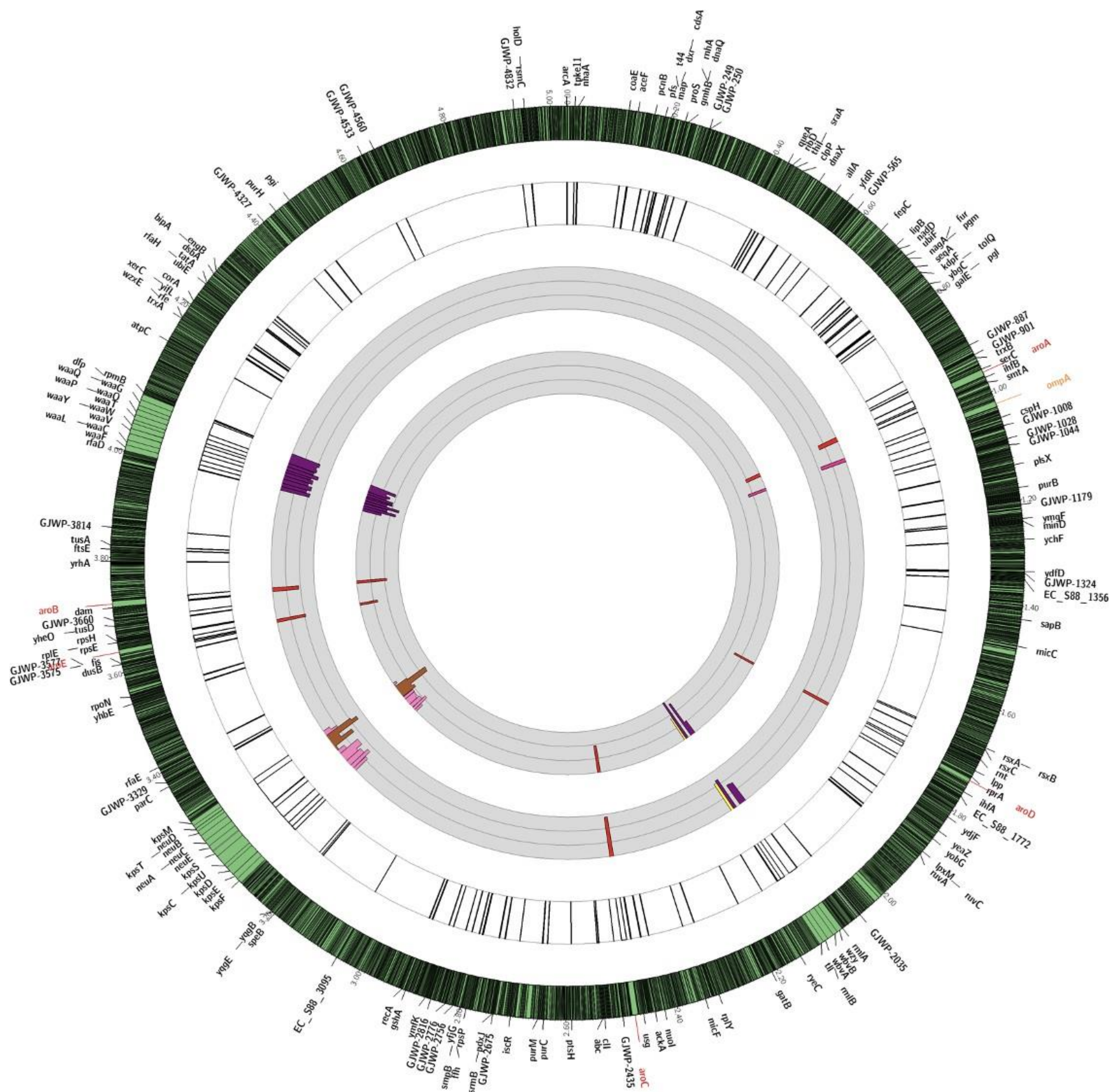


Figure S7. Important genes for systemic dissemination: localization on *E. coli* K1 S88 chromosome and quantitative analysis

The outermost circle represents the full *E. coli* K1 S88 genome with a 30-times magnification of the regions of interest. The first gray circle represents the fold change of the reads of the Tn insertions from the LB to the Liver after IP challenge. The second gray circle represents the fold change of the reads of the Tn insertions from the LB to the Liver after gavage. The thin grey circular lines represent 10-fold-changes (i.e., at $\log_{10}$ scale). The limit of the fold change representation is 1000. Bars represent changes in individual genes. Bars pointing toward the circle's center represent Tn interrupted genes resulting in negative fitness. Major, previously described, virulence factors of *E. coli* K1 are highlighted in color: LPS and K1 capsule encoding genes as examples for genes clusters, OmpA as example for a unique gene and Aro encoding genes as example of several individual genes (i.e not in cluster) located on several and distanced part of the chromosome.

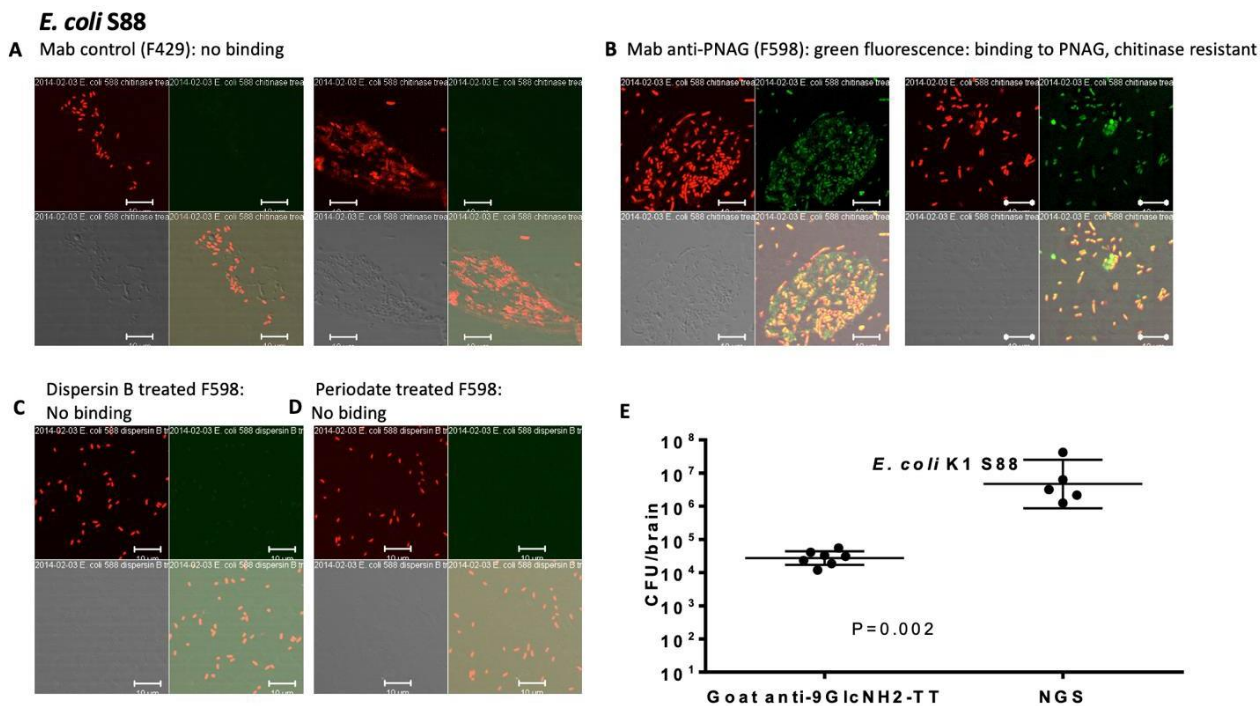


Figure S8. ***E. coli*** **K1 S88 and PNAG**

(A-D) Detection of PNAG production (green) by *E. coli* K1 using confocal microscopy to visualize binding of MAb F598 to PNAG. Control: MAb, F429, to *P. aeruginosa* alginate. The chemical properties of the PNAG antigen were confirmed using a previously reported approach by enzymatic digestion with Dispersin B (sensitive) and Chitinase (41). If PNAG is present, green fluorescence is dramatically decreased after Dispersin B treatments. Chemical specificity was confirmed using periodate (sensitive). (E) Prophylactic (24 h pre-challenge) effect of 50 µl of opsonic goat polyclonal antibodies to the synthetic oligosaccharide 9GlcNH2-TT on *E. coli* K1 levels in the brain 24 h after challenge by gavage. Controls received normal goat serum (NGS). Bars represent the mean, and error bars depict the 95% confidence interval. P values determined by nonparametric t-test.

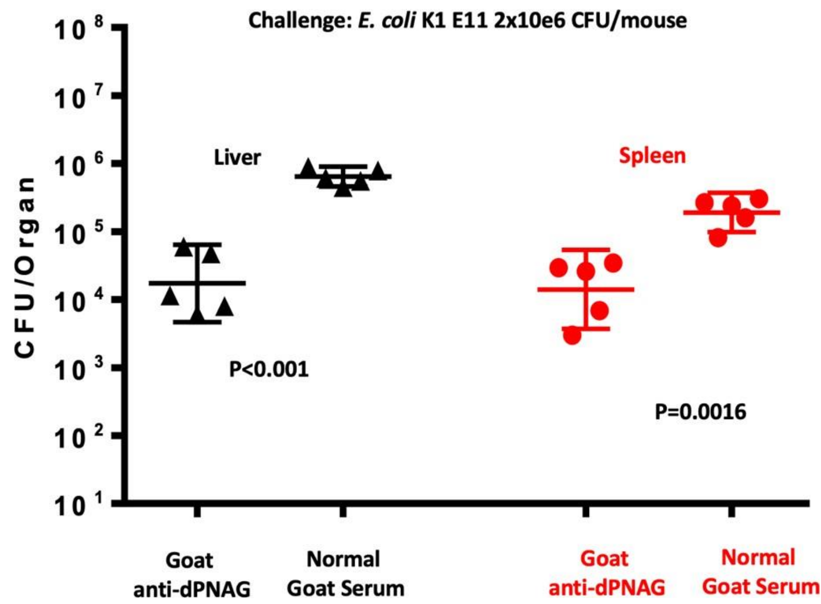
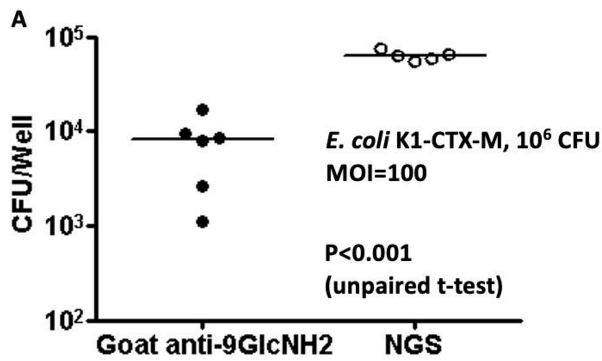
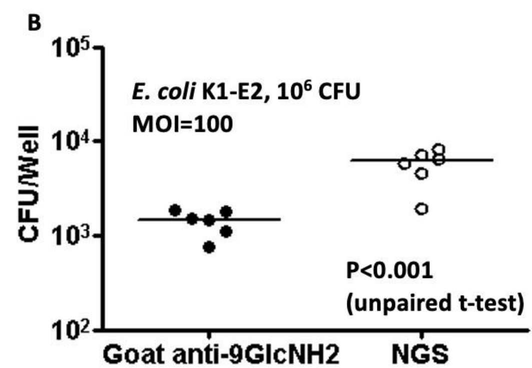


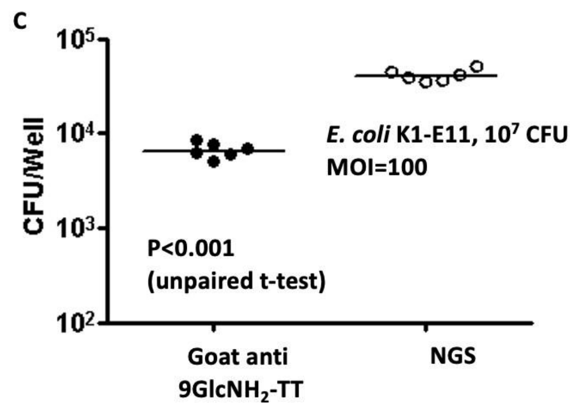
Figure S9. Prophylactic effect of polyclonal antibodies to PNAG on *E. coli* K1 levels in liver and spleen
Prophylactic (24 h pre-challenge) effect of 50 µl of opsonic goat polyclonal antibodies to PNAG on *E. coli* K1 levels in the liver and the spleen 24 h after challenge by gavage. Controls received normal goat serum (NGS). P values determined by nonparametric t-test. Bars represent the mean, and error bars depict the 95% confidence interval.

HBMEC Adhesion assay





HBMEC Invasion assay



HBMEC Translocation assay

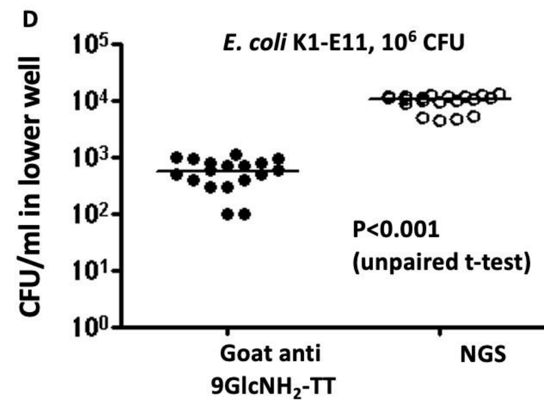


Figure S10. Adhesion (other strains), Invasion and translocation assays
Goat polyclonal antibodies to the synthetic oligosaccharide 9GlcNH2-TT significantly inhibits adherence, invasion and translocation of different strains of *E. coli* K1 applied to HBMEC compared to normal goat serum (NGS). (A) Adhesion assay of *E. coli* K1-CTX-M. (B) Adhesion assay of *E. coli* K1-E2. (C) Invasion assay of *E. coli* K1-E11. (D) Translocation assay of *E. coli* K1-E11. Symbols are individual wells, lines medians, P values unpaired t-tests.

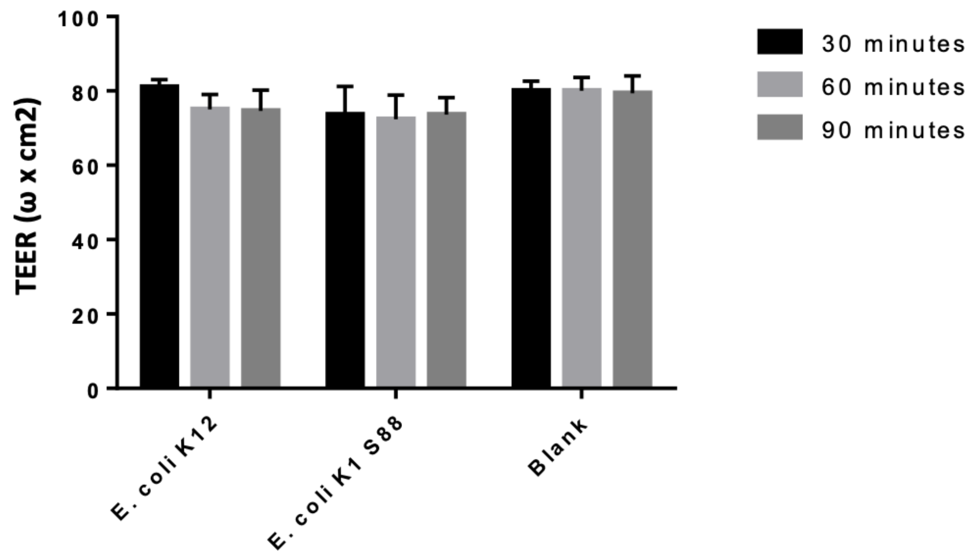


Fig. S11. Transendothelial electrical resistance of HBMEC in presence of different strains of *E. coli*
Transendothelial electrical resistance of HBMEC after 30, 60 and 90 minutes of incubation with *E. coli* K12, *E. coli* K1 S88 or blank. Bars represent the mean, and error bars depict the confidence interval

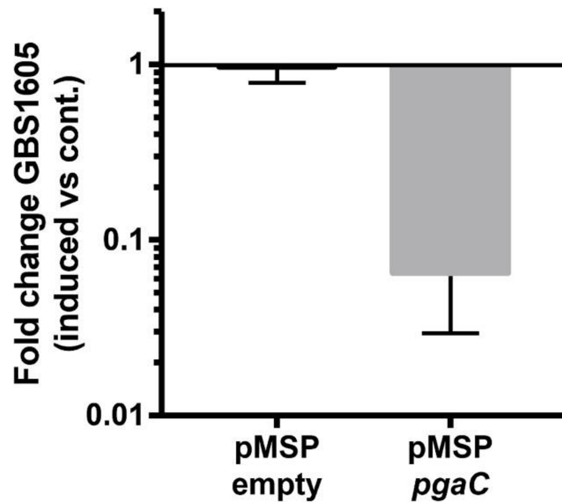


Figure S12. Decrease of GBS1605 transcripts in presence of nisin and asRNA pMSP*pgaC*
qRT-PCR reveals a 30-fold decrease in GBS1605 transcripts in the presence of 0·5 mg/mL nisin induction and the antisense construct pMSP*pgaC*. As a control, presence of empty vector pMSPempty had no significant effect on transcription levels of GBS1605 with or without nisin induction. Transcription changes are expressed in ratio induced versus non-induced. In all tested conditions, GBS1605 relative transcription levels were normalized using those of housekeeping gene tuf. Experiments were performed in three independent biological replicates. Bars represent the mean, and error bars depict the 95% confidence interval.

Table S1. Strains used in the study

Strain	Source
E. coli K1 S88	Provided by S. Bonacorsi[20]
E. coli K12 MG1655	
E. coli LF82	Provided by H. Sokol[21]
E. coli K1 E11	Clinical strain from the Brigham and Women's Hospital
E. coli K1 E11 Δ*pga*	This study
E. coli E2	Clinical strain from the Brigham and Women's Hospital
E. coli K1 CTX-M	Provided by R. Bonnet[22]
S. aureus PS80	Provided by J.C Lee
S. aureus USA300 LAC	[13]

Table S2. Plasmids and primers used in the study

Plasmid and primers	Relevant characteristics or sequence	Source or reference
Plasmid		
pRED/ET	Red/ED expression plasmid, Tet^R	[23]
pMSP3535	Nisin-inducible *PnisA* promoter	
pjRS33	Temperature-sensitive shuttle vector	
pCP20	FLP+, temperature-sensitive, Amp^R, Cm^R	Gene Bridges
Cassette		
FRT-PGK-gb2-neo-FRT	PGK-gb2-neo cassette flanked by FRT sites, Kan^R	[23]
Primers		
***E. coli* -pgaA-del5F**[a]	CTGTAATTAGATATAGAGAGAG ATTTTGGCAATACATGGAGTAAT ACAGG**AATTAAACCCTCACTA AAGGGCGG**	
***E. coli* -pgaD-del3R**[a]	ACTCACCAGCATCAGGATATATT TATTTCCATTACGTAACATATTTA TCC**TAATACGACTCACTATAGGG CTC**GTGTGTTATCGGTGCAGAGC CCGG	
***E. coli* -pgalocus-5'**	CGGATTATGAGGTGCAAAAA	
***E. coli* -pgalocus-3'**	TATCTTTCTTTTCAGTTACC	

Tet^R Tetracycline resistant, Amp^R Ampicillin resistant, Cm^R Chloramphenicol resistant, Kan^R Kanamycin resistant

[a] Boldface characters indicate the 20 nucleotides homologous to the FRT-PGK-gb2-neo-FRT cassette