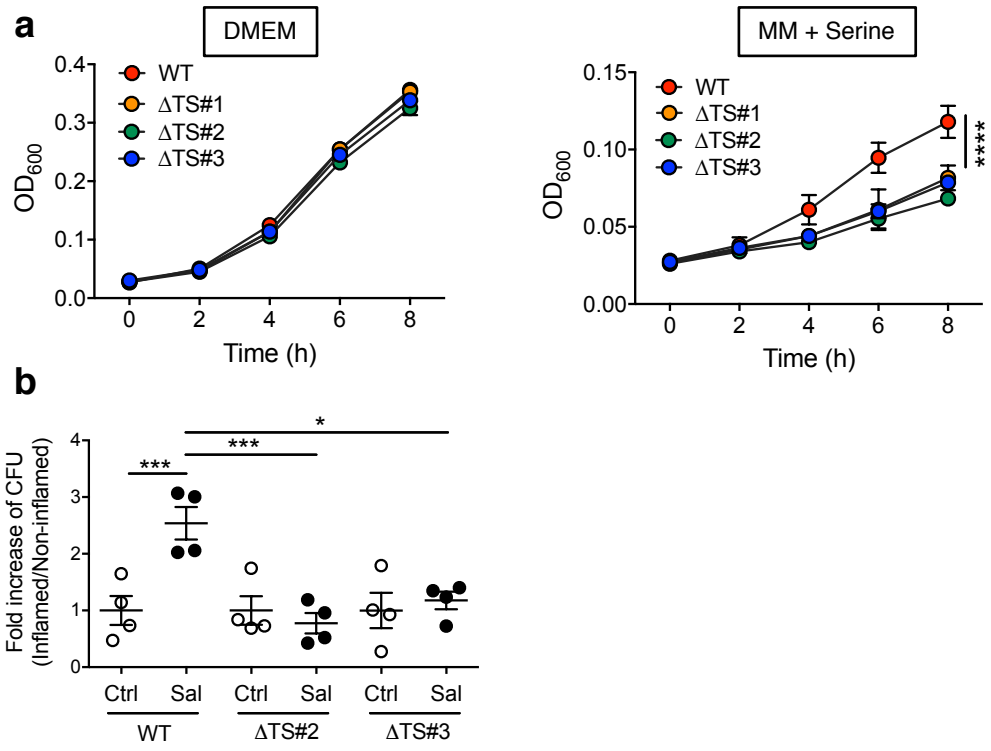

Supplementary figure 1. L-serine catabolism mutants show serine-dependent growth defect and impaired fitness in the inflamed gut.

(**a**) AIEC LF82 WT and Δ*tdc*Δ*sda* (ΔTS) mutant strain #1 (used in the main Figure and Extended Data Figures) and additional ΔTS double mutant strains #2 and #3 (generated in a clean background for validation) were cultured in DMEM (0.45% glucose) or a minimal medium (0.1% glucose) supplemented with 1 mM serine for 8 hours at 37ºC with 20% O_2 and 5% CO_2. Growth kinetics ($O.D_{600}$) are shown. Data represent geometric mean ± s.d. (N=3, biological replicates). ****; $P < 0.0001$ by 2-Way ANOVA. (**b**) AIEC LF82 WT and ΔTS mutants #2 and #3 (1×10^3 CFU) were inoculated into sterilized cecal content isolated from healthy and colitic (*Salmonella*-induced colitis) and cultured for 8 hrs at 37ºC with 20% O_2 and 5% CO_2. After 8hrs, culture media were plated onto LB agar and bacterial CFUs were measured. Fold increase of bacterial strains in the inflammation (+) (Sal) luminal content to the inflammation (-) control condition (Ctrl) is shown. Data represent mean ± s.d. (N=3, biological replicates). N.S.; not significant, *; $P < 0.05$, ***; $P < 0.001$ by Mann–Whitney *U* test (two-sided).

Table S3. Bacteria strains and plasmids used in this study.

Bacteria	Description	Reference or Source
MG1655	Human commensal *E. coli* (Str^R)	[25]
MG1655Δ*tdc*Δ*sda*	MG1655Δ*tdcABCDEFG* and Δ*sdaBC* (Amp^R, Kan^R, Cam^R)	This study
HS	Human commensal *E. coli* isolate (O9:H4); Carb	[24]
LF82	Human adherent-invasive *E. coli* isolate (O83:H1), Amp^R	[6]
LF82Δ*tdc*	LF82Δ*tdcABCDEFG* (Amp^R, Kan^R)	This study
LF82Δ*sda*	LF82ΔsdaBC (Amp^R, Cam^R)	This study
LF82Δ*tdc*Δ*sda*	LF82Δ*tdcABCDEFG* and Δ*sdaBC* (Amp^R, Kan^R, Cam^R)	This study
EcN	Human probiotic *E. coli* Nissle 1917, wild-type	Ardeypharm, Germany
CUMT8	Mouse adherent-invasive *E. coli* isolate	[23]
CUMT8 Str^R	Mouse adherent-invasive *E. coli* isolate (Str^R)	This study
E. coli SK460	Mouse commensal *E. coli* isolated from healthy gut	This study
E. coli dn15.6244.1	Mouse commensal *E. coli*	[26]
Citrobacter rodentium (DBS100)	Murine model of human EPEC or EHEC (Nal^R)	[46]
DBS100 Δ*tdc*Δ*sda*	*C. rodentium* DBS100 Δ*tdcABCDEFG* and Δ*sdaBC* (Nal^R, Kan^R, Cam^R)	This study
DH5α	*E. coli* used for plasmid amplification	Invitrogen

Plasmid	Description	Reference or Source
pKD46	Lambda Red recombinase expression plasmid	[39]
pKD3	Template plasmid for gene disruption (Cam^R)	[39]
pKD4	Template plasmid for gene disruption (Kan^R)	[39]

Table S4. Primers used in this study.

1. Primers for mutant construction		
Name	**Sequence**	**Reference**
tdcA_H1_F	TCATTTTGATACACTTCATACCGTCAATGAGGTAATTAACGTAGG TCGTTGTGTAGGCTGGAGCTGCTTC	This study
tdcG_H2_R	AAAAAAGGGTGCACATTTTGTGCACCCAAGAATGATAGCTGACA GCAATGATGGGAATTAGCCATGGTCC	This study
sdaC_H1_F	ATTAAGCCATCGCCGATAGACAGATTTCATTTTTACGGTCAGGC ACCTTCGTGTAGGCTGGAGCTGCTTC	This study
sdaB_H2_R	ATTCGATGCCAGAACAGGCTATGGCTGGATGAGAAATCGGGAA GAGGCCTATGGGAATTAGCCATGGTCC	This study
Screening_tdcA_F	CGATGCCATGGAAAGTATTG	This study
Screening_tdcG_R	CTCTATTTGCGGCAGATCAC	This study
Screening_sdaC_F	CTGATAGATACATAACTTGTGTGTATC	This study
Screening_sdaB_R	GCAAATGAACAGCCACGATAAC	This study
2. Primers for quantitative PCR		
Name	**Sequence**	**Reference**
tdcA_F	CCTGCCGGTCAATTGTTACTC	This study
tdcA_R	CCACCGCCTCAGAAGACATAC	This study
rrsB_F	TGTCGTCAGCTCGTGTTGTG	This study
rrsB_R	ATCCCCACCTTCCTCCAGTT	This study
3. Primers for bacteria quantification		
Name	**Sequence**	**Reference**
*Enterobacteriaceae*_F	GTGCCAGCMGCCGCGGTAA	[3]
Enterobacteriaceae _R	GCCTCAAGGGCACAACCTCCAAG	[3]
Eubacteria *16S*_F	ACTCCTACGGGAGGCAGCAGT	[3]
Eubacteria *16S*_R	ATTACCGCGGCTGCTGGC	[3]