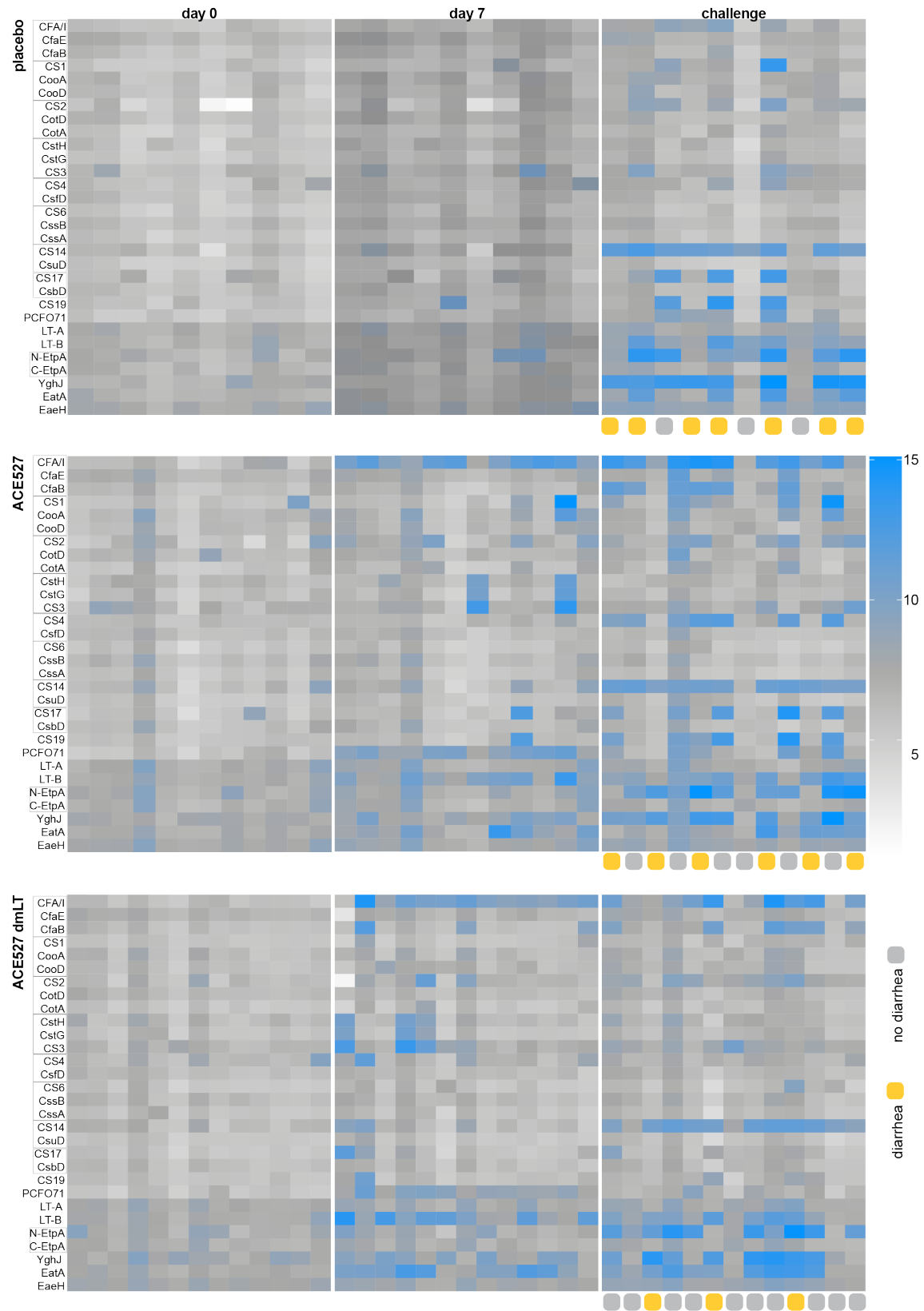
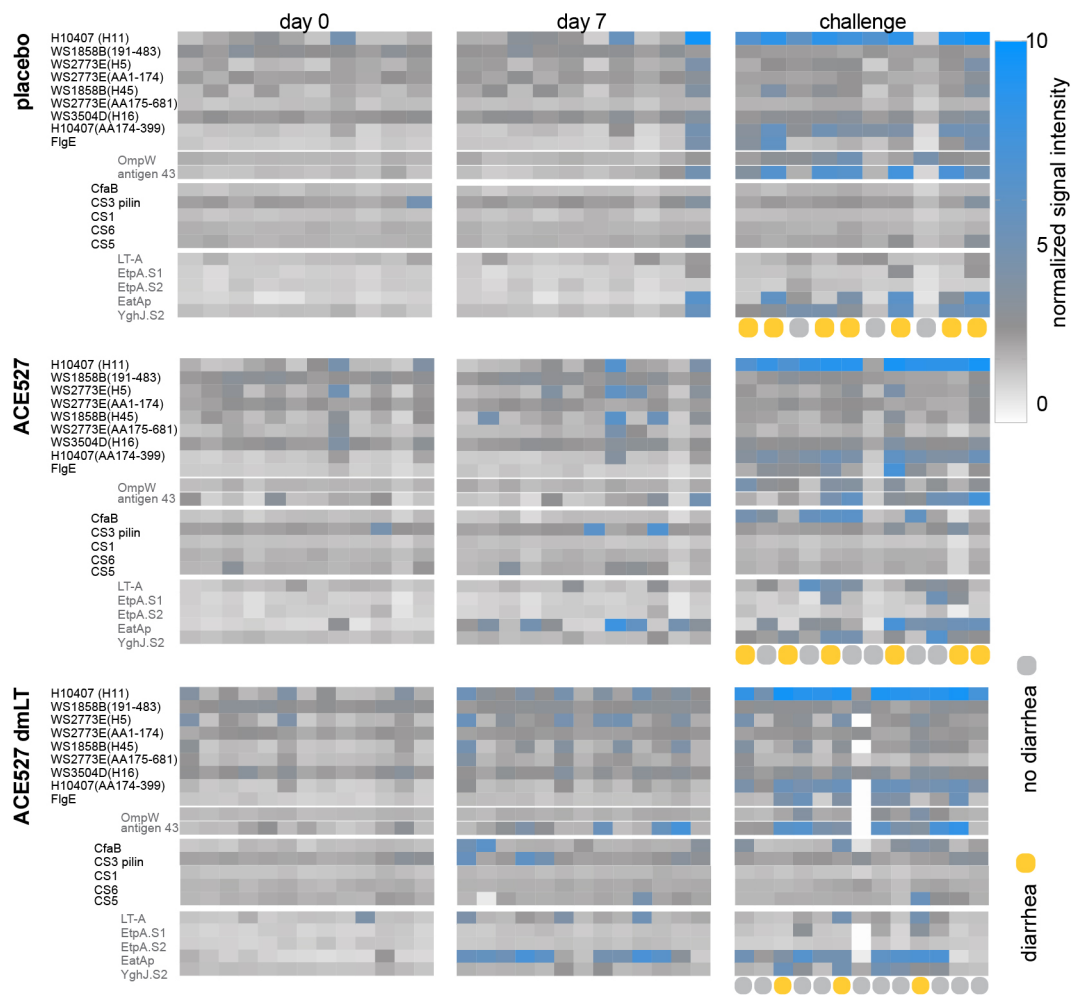


Supplementary figures



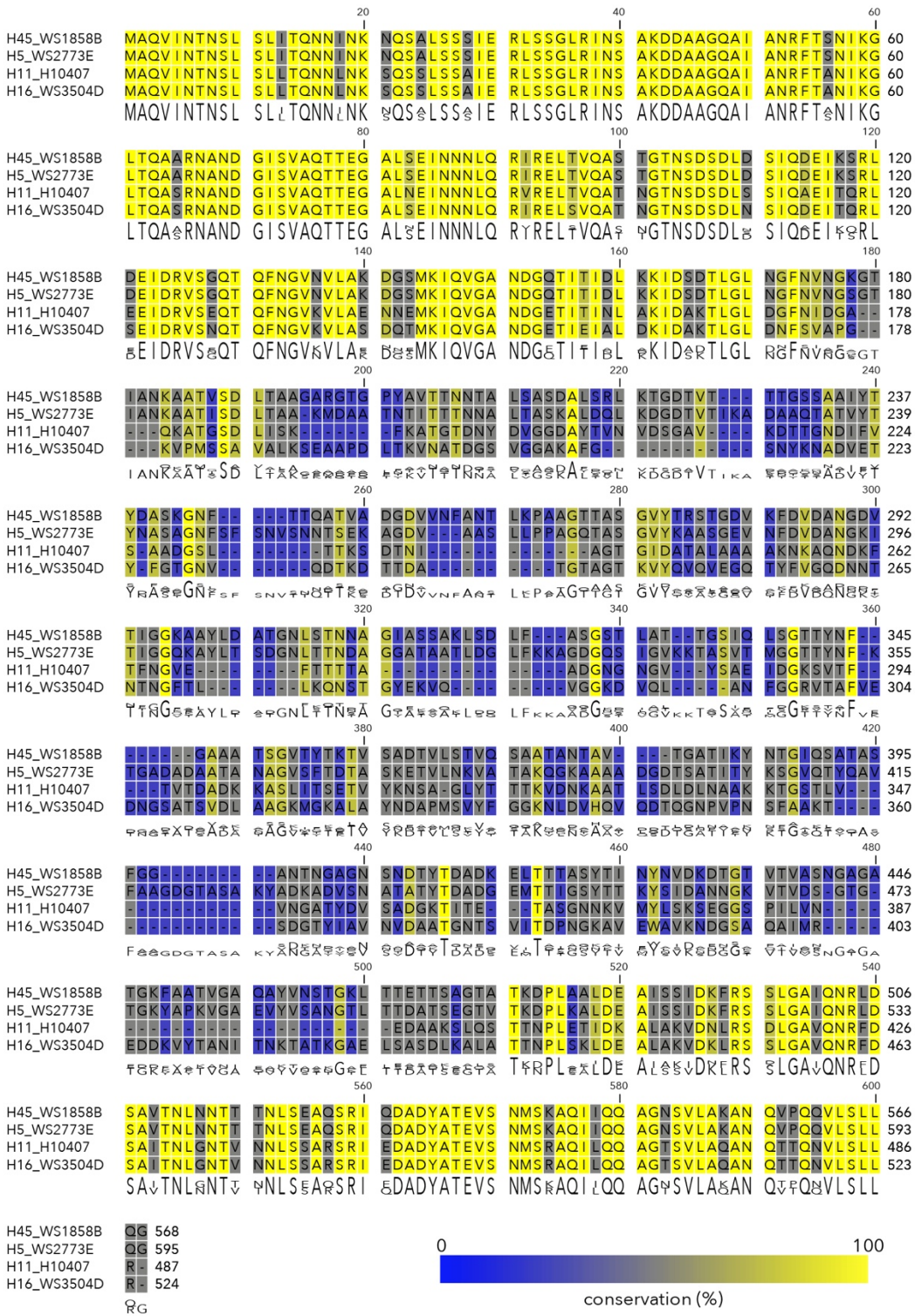
Supplementary figure 1

Antibody lymphocyte supernatant (ALS), IgA responses to recombinant antigens following vaccination with ACE527±dmLT, and challenge with H10407. Individual recombinant antigens are shown at left while individual volunteer data are arranged in columns. Data legend depicts log₂ values of array signal intensity.



Supplementary figure 2.

Antibody lymphocyte supernatant (ALS) IgA responses to select IVTT antigens following vaccination with ACE527, ACE527 + dmLT, or placebo. Shown are responses prior to vaccination, day 7 post vaccination, and day 7 post challenge with ETEC H10407. Antigens are grouped by row according to function (flagellar antigens at top; followed by membrane proteins OmpW, and antigen 43; CF/CS colonization factor antigens; secreted proteins at bottom). Columns in each group represent individual volunteers (placebo n=10; ACE527 n=12; ACE527 +dmLT n=13). Volunteers experiencing diarrhea after challenge with ETEC H10407 are represented as yellow circles while those without diarrhea post challenge are shown as grey circles. Legend values at right indicate normalized signal intensity.



Supplementary figure 3.

MUSCLE[7] alignment of H10407 flagellin (H11 sequence) with flagellin (FlIC) peptides from parental ACE527 strains WS1858B (H45), WS2773E (H5), and WS3504D (H16). MUSCLE v3.8.31 is available at <http://www.drive5.com/muscle/>.

Supplementary tables

Supplementary table 1

Supplementary table 1. H10407 challenge, ACE527 parental and vaccine strains			
strain	genotype	description	reference
live attenuated vaccine parents and constructs			
WS-2773E	O141:H5 CS5 CS6 LT ST EAST1	(parental clinical isolate)	[1]
ACAM2022	O141:H5 CS5 CS6 Δ astA Δ eltAB Δ estA Δ ompF:: <i>Ptac-LTB</i> Δ aroC Δ phage	vaccine isolate from WS-2773E	[1]
WS-3504D	O39:H16 ^a CS2 CS3 LT ST EAST1 Ap ^r	parental clinical isolate	[1]
ACAM2027	O39:H16 CS2 CS3 Δ astA Δ eltAB Δ estA Δ ompC:: <i>CS1</i> Δ ompF:: <i>Ptac-LTB</i> Δ aroC	vaccine isolate from WS-3504D	[1]
WS-1858B	O71:H45 ^b CFA/I ST EAST1 Ap ^r Tp ^r Su ^r	parental clinical isolate	[2]
ACAM2025	O71:H45 CFA/I Δ astA Δ eltAB Δ estA Δ ompC Δ ompF:: <i>pLLTB</i>	vaccine isolate from WS-1858B	[1]
clinical challenge strain			
H10407	O78:H11 CFA/I LT/ST/EAST1	wild type-challenge strain	[3]
^a Predicted by DNA sequence; Original serotype H12. ^b Predicted by DNA sequence, original serotype H ⁻ . https://cge.cbs.dtu.dk/services/SerotypeFinder/ [4]			

supplementary table 2 primers used in these studies

primer designation	sequence 5'-3'	description	amplicon size (bp)	Reference:
jf082213.1	ATGTGCTTTG GCAGGTTAAT	<i>eatA</i> forward primer	1934	[5]
jf082213.2	ATATCCAGTC AGCACCCACT	<i>eatA</i> reverse primer	1934	[5]
jf082213.3	GGTTCAGGCA GTATCCAGAC	<i>etpA</i> forward primer	999	[5]
jf082213.4	GGTGTAGCTG TCTGACCACA	<i>etpA</i> reverse primer	999	[5]
jf092313.7	TACAAGCAGGATTACAACAC	ST-H <u>reverse</u> primer bp 205-186 of st1b gene	64	[6]
jf092313.8	AGTGGTCCTG AAAGCATG	ST-H forward primer bp 146-159 of st1b	64	[6]
jf092313.5	TCTTTCCCCTCTTTTAGTCAG	ST-P forward primer	166	[6]
jf092313.6	ACAGGCAGGA TTACAACAAA G	ST-P reverse primer	166	[6]
jf092313.3	ACGGCGTTAC TATCCTCTC	LT-forward primer binds 31-49 of LT-B gene	274	[6]
jf092313.4	TGGTCTCGGTCAGATATGTG	LT-reverse primer binds 304-285 of LT-B gene	274	[6]

Supplementary table 3 genetic composition of ACE527 vaccine strains and parents

Supplementary table 3. genetic composition of ACE527 vaccine strains and parents ¹						
gene/locus	WS2773E	ACAM2022	WS1858B	ACAM2025	WS3504D	ACAM2027
colonization factors						
<i>cfaA</i>			+	+		
<i>cfaB</i>			+	+		
CS1						+
CS2					+	+
CS3					+	+
CS5	+	+				
CS6	+	+				
CS21			+	+	+	
toxins						
<i>eltA</i>	+				+	
<i>eltB</i>	+	+		+	+	+
<i>estH</i>	+				+	
<i>astA</i>	+				+	
<i>estP</i>						
secreted virulence antigens						
<i>etpBAC</i>			+		+	
<i>eatA</i>	+	+	+		+	+
<i>yghJ</i>	+	+	+	+	+	+
<i>cexE</i>			+	+		
<i>clyA</i>	+	+	+	+	+	+
cell surface antigens						
<i>tia</i>						
<i>tibA</i>						
<i>fimH</i>	+	+	+	+		
<i>fimA</i>	+	+	+	+		
<i>ecpA</i>	+	+				
antigen 43	+	+	+	+	+	+
motility						
<i>fliC</i>	+	+	+	+	+	+
<i>flgE</i>	+	+	+	+	+	+
toxin secretion						
T2SS ² (Gsp)	+	+	+	+	+	+
<i>toIC</i>	+	+	+	+	+	+

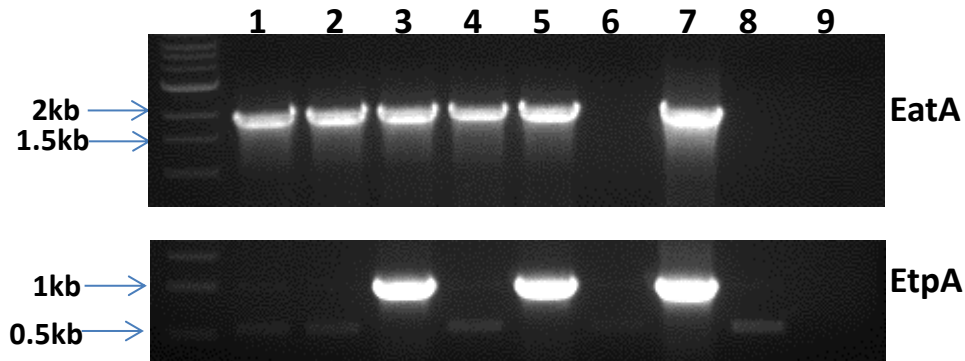
¹from whole genome sequence analysis of ACE527 parents and vaccine strains using RASTtk [28]

²T2SS = type 2 secretion system General Secretion Pathway genes *pppA* through *gspM*

Supplementary references

1. Turner AK, Stephens JC, Beavis JC, et al. Generation and characterization of a live attenuated enterotoxigenic *Escherichia coli* combination vaccine expressing six colonization factors and heat-labile toxin subunit B. *Clin Vaccine Immunol* **2011**; 18:2128-35.
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3. Evans DJ, Jr., Evans DG. Three characteristics associated with enterotoxigenic *Escherichia coli* isolated from man. *Infect Immun* **1973**; 8:322-8.
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6. Rodas C, Iniguez V, Qadri F, Wiklund G, Svennerholm AM, Sjoling A. Development of multiplex PCR assays for detection of enterotoxigenic *Escherichia coli* colonization factors and toxins. *J Clin Microbiol* **2009**; 47:1218-20.
7. Edgar RC. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* **2004**; 5:113.

PCR Results for EatA and EtpA



Well #	jf #	Sample ID	Well #	jf #	Sample ID
1	2864	WS2773E	6	2904	ACAM2025
2	2900	ACAM2022	7	H10407	Positive control
3	2866	WS3504D	8	MG-1655	Negative control
4	2902	ACAM2027	9	Water	Negative control
5	2862	WS1858B			

DNA template: genomic DNA

Primers: EatA (082213. 1&2) and EtpA (082213. 3&4)

Products' size: EatA=1934bp EtpA=999bp

8/27-28/18

