

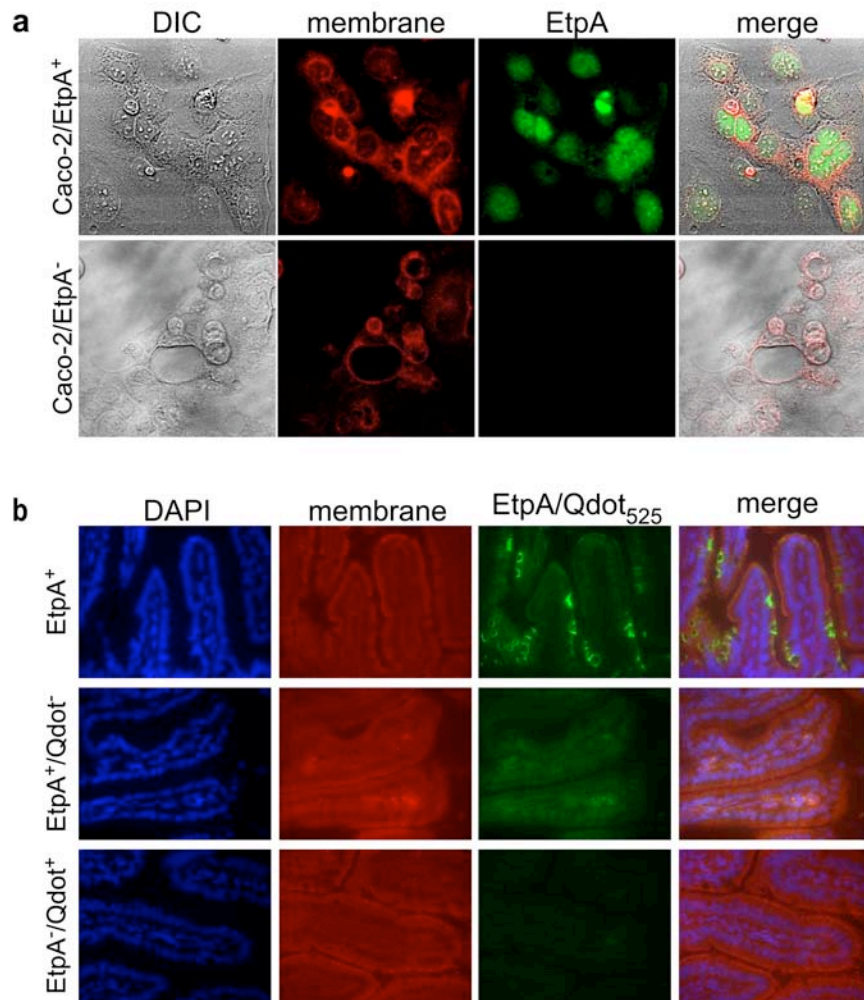
Supplementary Figure 1 | Role of EtpA and flagella in ETEC adherence.

a, Affinity-purified antibodies against flagellin (H48) inhibit ETEC H10407 (O78:H11) adherence to intestinal epithelial cells. (nrs = normal preimmune rabbit sera). **b**,

Antibody* directed at serotype-specific regions of flagellin has no effect on adherence. As shown at left, crude antisera vs full-length [1-498] H48 flagellin, as well as affinity-

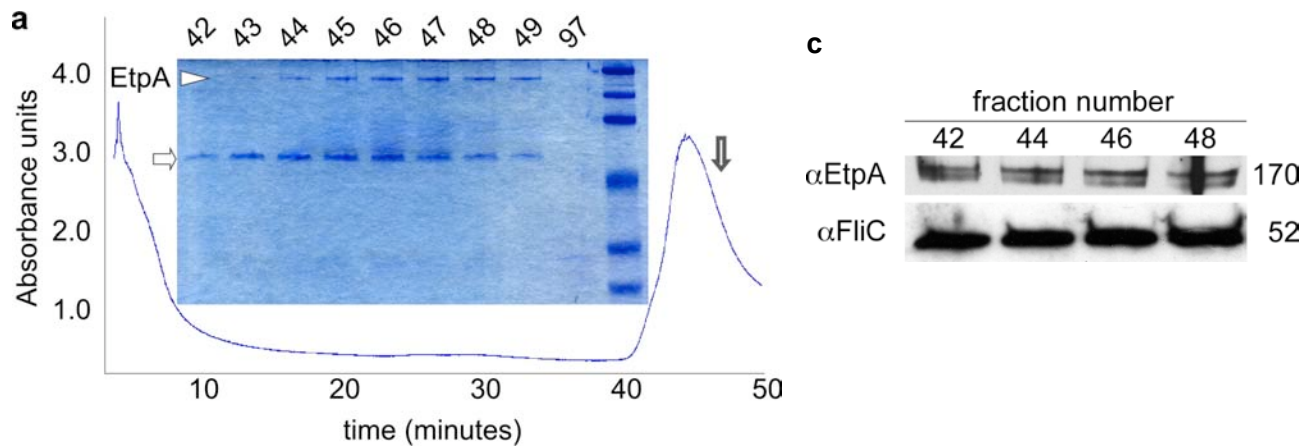
purified antisera against H48_[1-498] inhibited adherence of H10407; whereas antibodies recognizing either of the serotype-specific regions (H48_[173-399], H11_[174-385]) had no effect. Inset: Western blot to verify that antibody affinity-purified against serotype-specific region of H48 (H48_[173-399]), retains ability to recognize this antigen, but not the polyhistidine-tagged H11 recombinant, H11_[174-385]. Control lane at right contains same antigen (rFliC-H11_[174-385]-6His) probed with anti-6His antibody. **c**, Anti-EtpA antisera* inhibits adherence of multiple motile EtpA-producing ETEC strains H10407 (CFAI, O78:H11), DS61-1 (CFAII, O6:H16), and Tx-1 (CFAI, O78:H12). Relative amounts of EtpA produced by the respective strains are indicated in the anti-EtpA immunoblot (inset). [densitometry measurements by [ImageJ](#): H10407=61445, DS61-1=5957, Tx-1=33863]. Flagellin (FliC) is shown here as a loading control. **d**, addition of exogenous rEtpA to *etpA* mutant restores adherence in dose-dependent fashion.

*Neither antibody against EtpA or flagellin in these experiments could be shown to inhibit motility (in soft agar assays following mixing of bacteria with antibody); likewise we could not demonstrate that under the conditions used here that the antibodies led to clumping of the bacteria or that they affected growth of the organisms, suggesting that the effect on adherence is specific.



Supplementary figure 2 | EtpA interacts with intestinal surface molecules.

a, Recombinant EtpA bound to the surface of Caco-2 intestinal epithelial cells following incubation of cells with rEtpA purified from supernatants of EtpA-expressing Top10(pJY019) as shown by confocal immunofluorescence imaging. EtpA-negative controls (row 2) demonstrate labelling of cells with plasma membrane marker (CellMask, Invitrogen), but not EtpA (primary and secondary antibody concentrations are the same as in row 1). **b**, EtpA binds to murine intestinal mucosa. Frozen sections from mouse intestine incubated with biotinylated EtpA followed by detection with streptavidin-coated quantum dots (Qdot525, green). Cell membranes labelled with CellMask plasma membrane marker (red), and nuclei with DAPI (blue). (In control, row 2, red filter has been removed to demonstrate amount of bleed-through red fluorescence).



b. Search Result for MALDI-TOF data

Rank	Probability	Est. Z Score	Protein ID	%Cov.	pI	kDa
1	1.0	2.40	gij41651 emb CAA35488.1 hsg48 (E. coli)	42	4.5	51.29

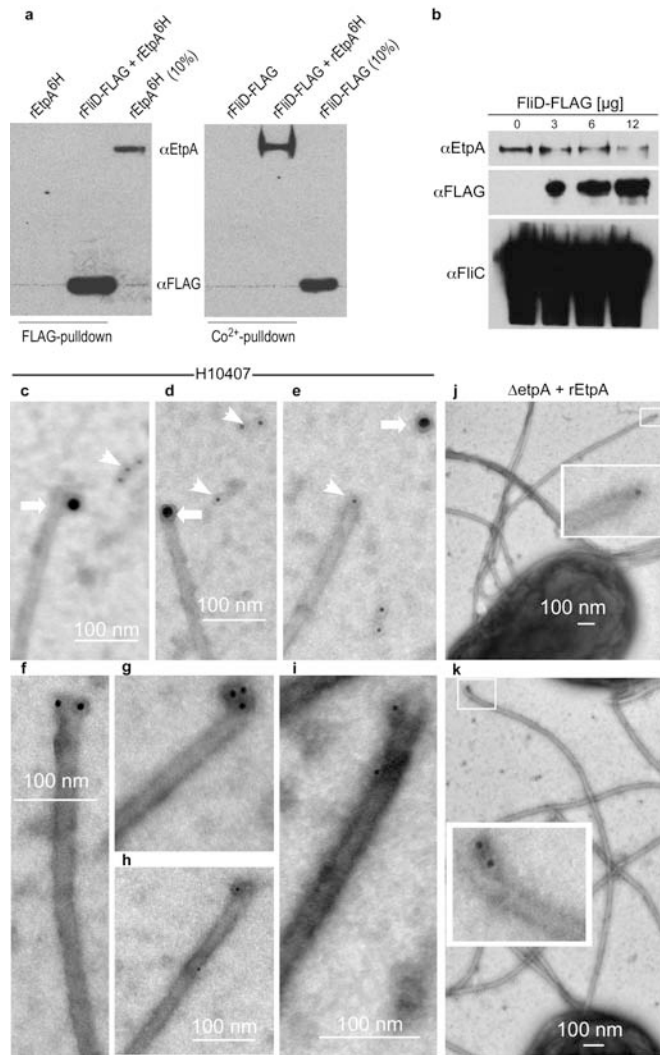
Mass and Sequence Detail for the 14 matched peptides to hsg48.

	Measured Mass (Da)	Computed Mass (Da)	Error (ppm)	Residues	Peptide sequence
1	1035.511	1035.559	-47	167-176	TLGLDGFSVK
2	1190.589	1190.589	0	21-31	NQSALSSSIER
3	1419.754	1419.735	13	413-425	ALDDAIASVDKFR
4	1439.815	1439.809	4	471-484	AQIIQQAGNSVLAK
5	1560.807	1560.825	-12	126-140	VSGQTQFNGVNVLAK
6	1670.757	1670.745	7	456-470	IQDADYATEVSNM*SK
7	1686.759	1686.740	11	456-470	IQDADYATEVSNM*SK
8	1741.900	1741.920	-11	146-161	IQVGANDNQITIDLK
9	1755.846	1755.852	-3	343-360	TITYTDSSGAASSPTAVK
10	2137.971	2138.012	-19	322-342	DTNGNLYAADVNETTGAVSVK
11	2248.092	2248.092	0	435-455	LDSAVTNLNNNTTTLSEASQR
12	2302.179	2302.191	-5	120-140	LDEIDRVSGQTQFNGVNVLAK
13	2337.137	2337.144	-3	177-199	NNDTVTTSAPVTAFGATTTNNIK
14	2628.268	2628.273	-2	67-91	NANDGISVAQTTEGALSEINNNLQR

*mass difference (approximately 16 Da) between peptides 6 and 7 is likely due to oxidized methionine residue at position 468 in peptide 7).

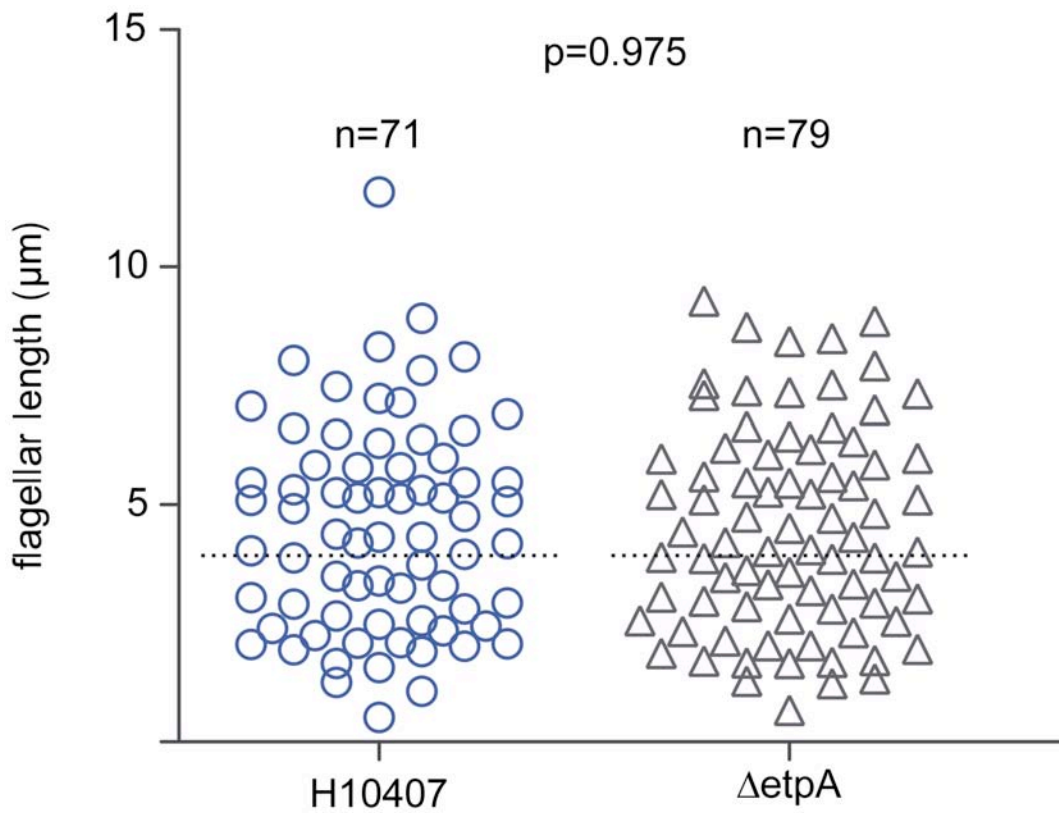
Supplementary Figure 3 | co-purification of EtpA and flagellins

a, Co-purification via gel filtration column chromatography of EtpA (arrowhead) and second (~50 kDa) protein (arrow) from supernatant of recombinant *E. coli* (H48) expressing the ETEC *etpBAC* TPS locus. Numbers above gel represent fractions collected at flow rate of 1 ml/min (therefore correspond to timeline at bottom of the chromatogram). Downward arrow indicates point of elution for standard (thyroglobulin, 670 kDa) under same conditions. **b**, MALDI-TOF identification of the ~50 kD co-purifying protein as flagellin serotype H48 (accession number [CAA35488](https://www.ncbi.nlm.nih.gov/Tran/TranQuery.fcgi?db=protein)) **c**, immunoblots of gel filtration-purified H10407 supernatants showing co-purification of EtpA with FliC (H11).



Supplementary figure 4 | EtpA binds to conserved regions of flagellin at flagellar tips when the FliD cap protein is absent.

a, Molecular pull down assays with EtpA and FliD protein demonstrate no apparent interaction between EtpA and FliD. Last lane in each immunoblot contains 10% of the protein used in the protein interaction studies as a positive control. **b**, FliD inhibits binding of EtpA to FliC-coated beads in molecular pull-down assays. **c-i**, Co-labelling experiments using primary antibodies against EtpA and FliD or conserved regions of FliC and FliD. **c-e**, EtpA (labelled with anti-mouse 20 nm gold conjugate (arrow) or FliD (labelled with 10 nm anti-rabbit conjugate, arrowheads) identified at the tips of flagella. Out of 42 intact flagella examined, 11 (26%) were labelled with EtpA alone, while 15 (36%) labelled with FliD alone. **f-g**, Labelling of FliD (10 nm anti-mouse gold conjugate), or **h-i**, conserved regions of FliC (1-173) (5 nm anti-rabbit gold conjugate). **j-k**, rEtpA added exogenously to the *etpA* mutant localizes to the tips of flagella. [final EtpA concentrations are $\approx 3 \mu\text{g/ml}$ in **j**, and $15 \mu\text{g/ml}$ in **k**]. (Here anti-myc primary antibody was used to recognize the tag on the end of rEtpA).



Supplementary figure 5 | EtpA does not compromise flagellar length.

Examination of WT and *etpA* mutant strains revealed no appreciable difference in motility or length of the flagella, indicating that EtpA normally attaches to the tips in a way that does not interfere with flagellar maturation. Sizes were determined by measuring flagella [in transmission electron microscopy images] of either the H10407 parent ETEC strain or the *etpA* mutant grown in liquid culture and fixed on grids.

