

SUPPLEMENTARY ONLINE DATA

Recruitment and membrane interactions of host cell proteins during attachment of enteropathogenic and enterohaemorrhagic *Escherichia coli*

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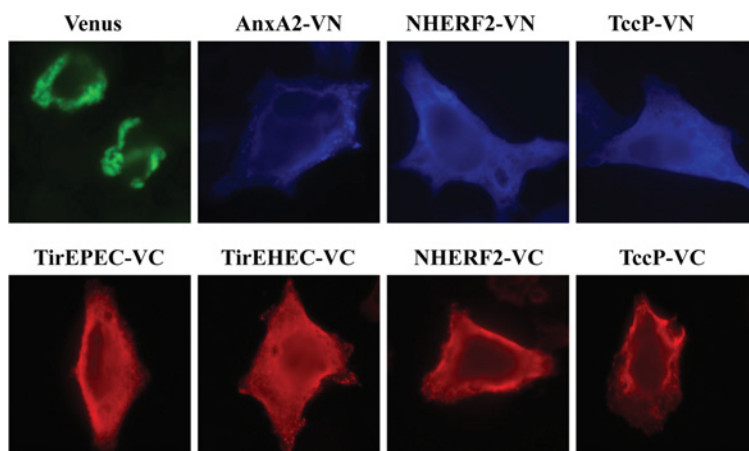


Figure S1 Expression of BiFC constructs in HeLa cells

Fluorescence microscopy of HeLa cells transfected with Venus protein or BiFC constructs. The green GFP signal in cells transfected with pVenus corresponds to Venus protein. AnxA2–VN was stained with mouse anti–Myc antibodies and NHERF2–VN and TccP–VN were stained with mouse anti–FLAG antibodies, followed by Cy5 (indodicarbocyanine)-conjugated anti–mouse antibodies (blue). Tir–EPEC–VC, Tir–EHEC–VC, NHERF2–VC and TccP–VC were stained with TRITC (tetramethylrhodamine β -isothiocyanate)-conjugated anti–HA (red).

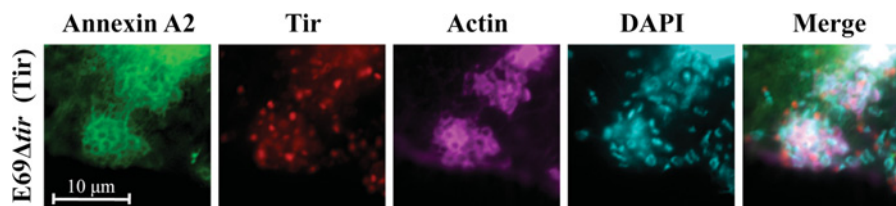


Figure S2 AnxA2 is recruited during infection of EPEC Δ tir complemented with a plasmid encoding Tir

Fluorescence microscopy of HeLa cells transfected with GFP–AnxA2 and infected with EPEC Δ tir containing pSA10 encoding Tir. The green GFP signal corresponds to AnxA2, Tir was stained with rabbit anti–Tir followed by Cy3-conjugated anti–rabbit antibodies (red), actin was stained with Alexa Fluor® 647–phalloidin (magenta) and DNA was stained with DAPI (cyan). AnxA2's recruitment is restored during infection of the E69 Δ tir strain when complemented with plasmid-encoded Tir.

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Table S1 Sequences of primers used in the present study

Restriction sites are in bold.

Primer	Sequence
Fw-BamH1-AnxA2	5'-CTG GGATCC ATGTCTACTGTTACGAAATCCTG-3'
Rv-AnxA2-BamHI	5'-CCG GGATCC TAGTCATCTCCACCACACAGGTAC-3'
SacI-Myc-VN173-For	5'-AAGCAG AGCTCG TTTAGTGAACCGTCAGAATTGATCTACCATGGAGCAGAAGCTGATCTCCGAGGAGGACCTGGGAGCAGTGAGCAAGGGCGAGGAGCTGTTCA-3'
VN-Anx-BamH1-Rev	5'-ACCCG GGATCC TAGTCATCTCCACCACACAGGT-3'
Fw-EcoRI-Nherf2-VN	5'-G CGAATTC GATGGCCGCGCCGGAGCCGCTGCG-3'
Rv-Nherf2-KpnI-VN	5'-ACT GGTACC GAGAAAGTTGCTGAAGATTCACGC-3'
Fw-EcoRI-Nherf2-VC	5'-CC GAATTC GGATGGCCGCGCCGGAGCCGCTGCG-3'
Rv-Nherf2-KpnI-VC	5'-ACG GGTACC GAAGTTGCTGAAGATTCACGC-3'
Fw-EcoRI-TccP-VN	5'-G CGAATTC GATGATTAACAATGTTTCTCA-3'
Rv-TccP-KpnI-VN	5'-ACT GGTACC GACGAGCGCTTAGATGATTAATGC-3'
Fw-TccP-EcoRI	5'-CC GAATTC GGATGATTAACAATGTTTCTCA-3'
Rv-TccP-VC-KpnI	5'-ACG GGTACC GAGCGCTTAGATGATTAAT-3'
Fw-Tir-EHEC-BglII	5'-ACCG AGATCT CTATGCCTATTGGTAATCTTGGT-3'
Rv-Tir-EHEC-KpnI	5'-ACG GGTACC GACGAAACGATGGGATCCCGGC-3'
Fw-Tir-EPEC-EcoRI	5'-CC GAATTC GGATGCCTATTGGTAACCTTGGTA-3'
Rv-TirVC-EPEC-KpnI	5'-ACG GGTACC AACGAAACGTAAGTGGTCCCGGC-3'
NcoI-Int280 γ	5'-CATG CCATGG ATATTAAGGCTGATAAGACAACTGC-3'
EcoRI-Int280 γ -Rv	5'-CG GAATTC GGTTCTACACAAACCGCATAGACATTTG-3'
XbaI-AnxA2	5'-GCCCT CTAGA AATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGTCTACTGTTACGAAATCCTGTG-3'
AnxA2-XhoI	5'-GTGGTG CTCGAG GTTCATCTCCACCACACAGGTACAGC-3'
AnxA2-C-HA-XhoI	5'-GTGGTG CTCGAG AGCGTAATCTGGAACATCGTATGGGTAGTCATCTCCACCACACAGGTACAGC-3'
AnxA2-N-HA-XhoI	5'-GTGGTG CTCGAG AGCGTAATCTGGAACATCGTATGGGTAGTCATCTCCACCACACAGGTACAGC-3'
XbaI-AnxA2-Cterm	5'-GCCCT CTAGA AATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGATTGACCAAGAT-3'
XbaI-Tir-Fw	5'-G CTCTAGA AATAATTTTGTTTAACTTTAAGAAGGAGATATAATGCCATTGGTAATCTTGGTTCAT-3'
XhoI-Tir-Rv	5'-CCG CTCGAG GACGAAACGATGGGATCCCGGCGC-3'
Fw-Xba-Tir-EPEC	5'-GCCCT CTAGA AATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGCCTATTGGTAACCTTGGTAATAATGTAAT-3'
TirC-EPEC-NotI-b	5'-GTGCTCGAGT GCGGCCG CAACGAAACGTAAGTGGTCCCGGCGTGGT-3'
Fw-Xba-Tir-Citrob	5'-GCCCT CTAGA AATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGCCTATTGGTAATCTTGGTAATAATAATAAG-3'
Rv-Tir-Citrob-NotI	5'-GTGCTCGAGT GCGGCCG GACGAAACGTTCAACTCCCGGTGTTGATGC-3'
XbaI-TirN	5'-GCCCT CTAGA AATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGCCTATTGGTAATCTTGGTTCATA-3'
TirN-XhoI	5'-GTGGT GCTCGAG AGTCCCAACGCCAACCAAGTTAG-3'
Rv-TirN-EPEC-NotI	5'-GTGCTCGAGT GCGGCCG CTGCGCCGACAGAAACCCAGAATTTAGGA-3'
Rv-TirN-Citrob-NotI	5'-GTGCTCGAGT GCGGCCG CTGCGCCGACAGAAACCCAGAATTTAGGA-3'
XbaI-TirC	5'-GCCCT CTAGA AATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGAGTGGCGCATGATTCTTGGTGGGGGA-3'
TirC-XhoI	5'-GTGGT GCTCGAG GACGAAACGATGGGATCCCGGCGCTG-3'
XbaI-TirC-EPEC	5'-GCCCT CTAGA AATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGAGCAGTGCATTGATTGTTGCTGGGGGA-3'
XbaI-TirC-Citrob	5'-GCCCT CTAGA AATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGAGTAGTGCATTGATTGTTGTTGGTGGGA-3'
TirC-Citrob-NotI-b	5'-GTGCTCGAGT GCGGCCG GACGAAACGTTCAACTCCCGGTGTTGTA-3'

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