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Spa32 interaction with the inner-membrane Spa40 component of the type III secretion system of *Shigella flexneri* is required for the control of the needle length by a molecular tape measure mechanism

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SUPPLEMENTARY MATERIAL

Table S1. List of strains and plasmids used in this study.

Strains	Relevant features	Reference
M90T-Sm	<i>S.flexneri</i> strain is a derivative of the wild type strain M90T (serotype 5) which is resistant to streptomycin	Allaoui et al. (1992)
$\Delta spa32$	<i>spa32</i> mutant (pWR100- <i>spa32::aphA-3</i>)	Magdalena et al. (2002)
Plasmids	Relevant features	Source
pMJ8	pQE30 carrying the entire <i>spa32</i> gene (His-Spa32)	Magdalena et al. (2002)
pAB34	pQE30- <i>spa32</i> Δ_{2-10} (His-Spa32 lacking the first 9 residues: D1)	This study
pAB36	pQE30- <i>spa32</i> Δ_{2-36} (His-Spa32 lacking the first 36 residues: D2)	This study
pAB35	pQE30- <i>spa32</i> Δ_{36-94} (Internal deletion within His-Spa32: D3)	This study
pAB38	pQE30- <i>spa32</i> Δ_{94-187} (Internal deletion within His-Spa32: D4)	This study
pAB37	pQE30- <i>spa32</i> Δ_{94-159} (Internal deletion within His- Spa32: D5)	This study
pAB39	pQE30- <i>spa32</i> $\Delta_{130-187}$ (Internal deletion within Spa32: D6)	This study
pAB47	pQE30- <i>spa32</i> $\Delta_{130-159}$ (Internal deletion within Spa32: D7)	This study
pAB46	pQE30- <i>spa32</i> $\Delta_{206-280}$ (Internal deletion within Spa32: D8)	This study
pAB65	pQE-30- <i>spa32</i> $\Delta_{246-292}$ (Spa32 deleted from the T3S4 domain: D9)	This study
pAB66	pQE30- <i>spa32</i> $\Delta_{263-292}$ (Internal deletion within Spa32: D10)	This study
pAB95	pQE30- <i>spa32</i> $\Delta_{206-246}$ (Internal deletion within Spa32: D11)	This study
pAB51	pGEX4T1-Spa40CT (GST-Spa40CT : a GST fusion with the 142 C-terminus residues of Spa40)	This study
pDR1	pTZ18R vector expressing InvJ of <i>Salmonella</i>	This study
pAB75	pTZ18R vector expressing YscP of <i>Y.enterocolitica</i>	This study
pAB106	pTOPO vector expressing Spa32 ₁₋₁₄₅ -YscP ₂₀₂₋₃₀₆ -Spa32 ₁₄₆₋₂₉₂ chimeric protein (Sopa32 Ω)	This study
pAB103	pTZ18R expressing His-YscP	This study
pAB104	pTZ18R expressing His-InvJ	This study
pCAK-P	pQE60 expressing YscP-His	This study
pCAK-J	pQE60 expressing InvJ-His	This study

SUPPLEMENTARY MATERIAL

Table S2. List of primers used in this study.

Primers	Sequence	Restriction site
InvJs	5'- GCGAATT CTATATCCAGCGAGAGAT -3'	<i>EcoRI</i>
InvJas	5'- TCCCC CGGG ATCAATCTGTCTCACACG -3'	<i>SmaI</i>
YscPs	5'- GCGAATTC ATTAT CAGGAAGAAC -3'	<i>EcoRI</i>
YscPas	5'- TCCCC CGGGG CGTTGTAATCGCTCAAG-3'	<i>SmaI</i>
Spa40-15	5'- AGGATCC GATAAACAGGAGATAAAAAGAGA-3'	<i>BamHI</i>
Spa40-16	5'- GTCGACC ATCTCCTTTACTTATTAATGAGTGTT-3'	<i>Sall</i>
InvJshisEI :	5'- TGGAATTC GCATCATCATCATCATCATGGCGATGTGTC AGCTGTCAGT-3'	<i>EcoRI</i>
YscPshisEI :	5'- TGGAATTC GCATCATCATCATCATCATAATAAAATCAC CACTCGTTCC-3'	<i>EcoRI</i>
<u>InvJsNc</u> :	5'- AT ACCATGGG CGATGTGTCAGCTGTCA-3'	<i>NcoI</i>
<u>InvJasBg</u>	5'- ATGAGATCTGGCGTCATCCTCCTCGCC-3'	<i>BglII</i>
<u>YscPsNc</u> :	5'-AT ACCATGGT GAATAAAAATCACCCTCGTTCCCCA-3'	<i>NcoI</i>
<u>YscPasBg</u>	5'- ATGAGAT CTTTCTT CAGCCTCCCCTCC-3'	<i>BglII</i>
YscPs202	5' -GAACCGTTACCTCTTCATCAA-3'	
YscPas302	5'-GTCGCGGTAGTTCGATG-3'	
5'P. primers^a		
Spa32-D1s	5'- AGTAGTGACAAACAGATAGAA -3'	
Spa32-D1as	5'- GGATCCATGGT GATGGT GATG -3'	
Spa32-D2s	5'- AGGAAGGTTGAAATTCCTGAG -3'	
Spa32-D2as	5'- GGATCCATGGT GATGGT GATG -3'	
Spa32-D3s	5'- AAAGAAAATATTTTGATACCA -3'	
Spa32-D3as	5'- CTTCTTCTTCAAACGAGACT -3'	
Spa32-D4s	5'- AATAAAAATACTGCAGACATA -3'	
Spa32-D4as	5'- AATGGCCATTGATTCTGGAAT -3'	
Spa32-D5s	5'- AGCTTGTCTATTGATAAG -3'	
Spa32-D5as	5'- AATGGCCATTGATTCTGGAAT -3'	
Spa32-D6s	5'- AATAAAAATACTGCAGACATA -3'	
Spa32-D6as	5'- CTTGTCTGCATT TCCAC -3'	
Spa32-D7s	5'-AGCTTGTCTATTGATAAG -3'	
Spa32-D7as	5'-CTTGTCTGCATT TCCAC -3'	
Spa32-D8s	5'- GTACAGGATATAATGAACAGAGTG -3'	
Spa32-D8as	5'- CTATTGATTTTTCTTGCAATGACA -3'	
Spa32-D9s	5'- TAAGAATTAAACATTTTGACG -3'	
Spa32-D9as	5'- CTCCACTGAAATACTAACAGA -3'	
Spa32-D10s	5'- ACGCTAACGAAAACTACAGA -3'	
Spa32-D10as	5'- AGCATCTTGT TTCAA AATTAAGTC -3'	
Spa32-D11s	5'- TCTGGTTCTTTTGTCTAAAACCGT -3'	
Spa32-D11as	5'- CTATTGATTTTTCTTGCAATGACA -3'	

Added restriction sites are indicated on Bold. ^a5' phosphorylated primers.

SUPPLEMENTARY MATERIAL

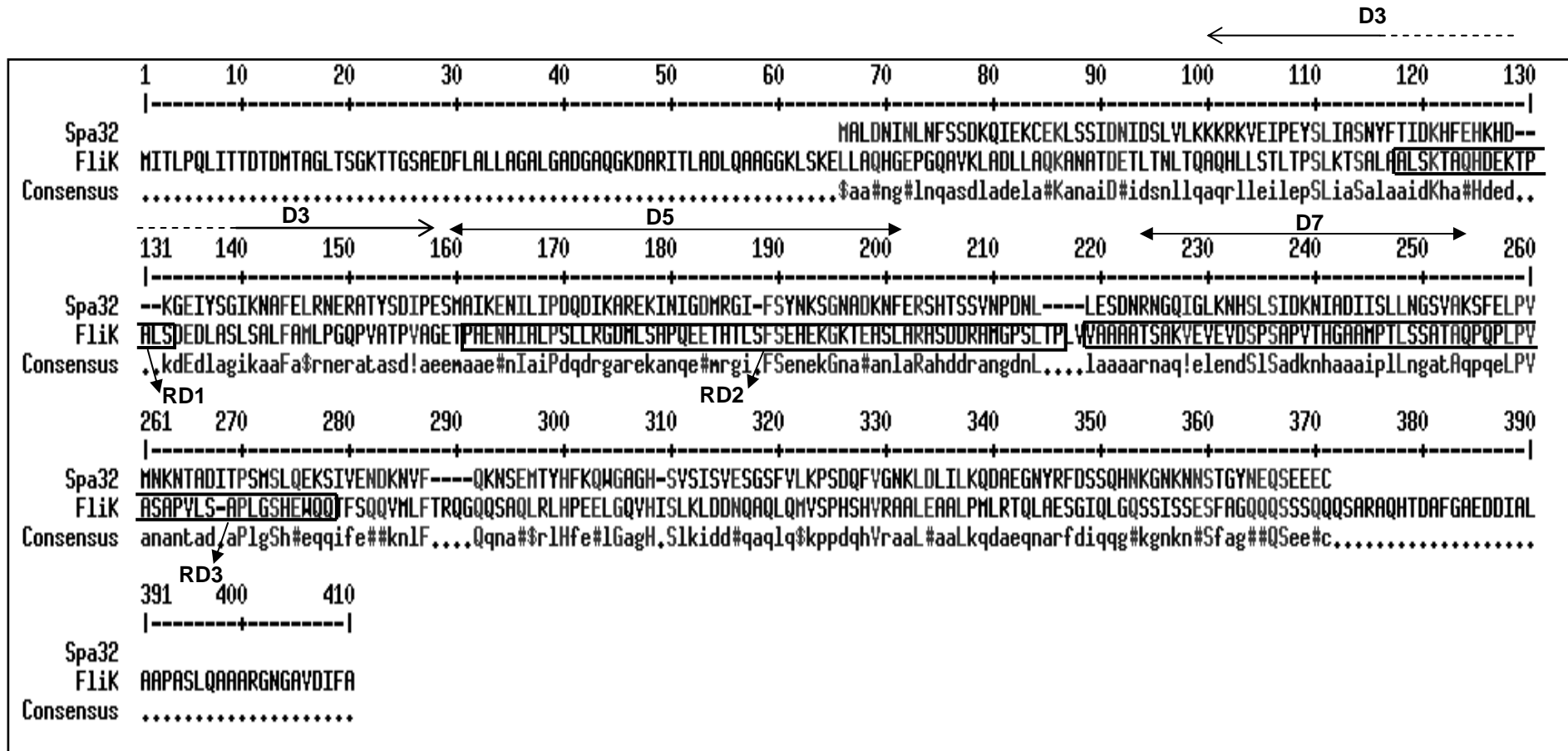


Figure S1. Sequence alignment of FliK and Spa32 protein. The three FliK ruler domains (RD) reported by Shibata et al. (2007) are indicated by open boxes: RD1: residues 118-133; RD2: residues 161-216, and RD3: residues 218-278. The positions of residues deleted from Spa32 in D3, D5, D6 and D7 proteins are indicated by horizontal arrows. The sequence of DR1 of FliK is entirely deleted in D3. DR2 domain of FliK is completely deleted in D5 and partly in D7 and D6. RD3 domain of FliK is partly deleted in D7 and mostly deleted in D6. In contrast to FliK variants lacking RD2 and RD3 that are still functional, D5 and D6 have lost the capacity to restore wild-type phenotype of the *spa32* mutant.