Supplemental material to Zilkenat S. et al. "Determination of the stoichiometry of the complete bacterial type III secretion needle complex using a combined quantitative proteomic approach"

Table S1: Strains and plasmids

Salmonella	**Description**	**Reference**
SB762	wild type (SL1344, flhD::tet)	(1)
SB1769	$SpaS_{N258A}^{FLAG}$, flhD::tet	(2)
SB1906	$InvA^{FLAG}$, flhD::tet	(2)
MIB3085	Δ*spaO*, flhD::tet	this study
Escherichia coli	**Description**	**Reference**
AT713	auxotroph Arg, Lys	(3)
Plasmid	**Description**	**Reference**
pSB3292	pBAD24, HilA	(4)
pMAL-c5x	vector for maltose binding protein fusion	New England Biolabs
pMIB5117	pUC57-PCS1	this study
pMIB5118	pMAL-c5x-PCS1	this study
pMIB5363	pMAL-c5x-PCS2	this study

References

1. Kaniga, K., Tucker, S., Trollinger, D., and Galan, J. E. (1995) Homologs of the Shigella IpaB and IpaC invasins are required for *Salmonella typhimurium* entry into cultured epithelial cells. *J Bacteriol* 177, 3965–3971

2. Wagner, S., Königsmaier, L., Lara-Tejero, M., Lefebre, M., Marlovits, T. C., and Galán, J. E. (2010) Organization and coordinated assembly of the type III secretion export apparatus. *Proc Natl Acad Sci USA* 107, 17745–17750

3. Taylor, A. L., and Trotter, C. D. (1967) Revised linkage map of Escherichia coli. *Bacteriol Rev* 31, 332–353

4. Lara-Tejero, M., Kato, J., Wagner, S., Liu, X., and Galán, J. E. (2011) A sorting platform determines the order of protein secretion in bacterial type III systems. *Science* 331, 1188–1191

Table S2: Oligonuceotides used for cloning of the peptide concatenated standards into pMAL-c5x

Oligonucleotide	Description	Sequence 5‘ – 3‘
gib_pMalc5x_QCAT4.1_f	cloning PCS2	CATGGGCGGCCGCGATATCGTCGACGGATCCCGCTTTCTGAAGAACAGTTTTG CAG
gib_pMalc5x_QCAT4.2_r	cloning PCS2	CTGAGCCTTTCGTTTTATTTGAAGCTTCGATCTAAGCGGCTTTCAGTACATGAG
gib_*QCAT4.1*_4.2_f	cloning PCS2	CTTGCATTGGCCAAAGGCGCCGGCAACATCAAAATTGTAGCCTATC
gib_QCAT4.2_4.1_r	cloning PCS2	GATAGGCTACAATTTTGATGTTGCCGGCGCCTTTGGCCAATGCAAG
gib_pMalc5x_f	cloning PCS1	AAGCTTCAAATAAAACGAAAGGCTCAG
gib_pMalc5x_r	cloning PCS1	GGATCCGTCGACGATATCGCGGCCGCCCATG
pMal_seqf	sequencing primer	GTCGTCAGACTGTCGATGAAG
pMal_seqr	sequencing primer	GATTTGTCCTACTCAGGAG
QCAT3_seq_1254_f	sequencing primer	GATCGTGAGCTTGTGCAG
QCAT3_set1_f	sequencing primer	CTAGGGATCCGCACGCTTGTATTCCGCAATTG
QCAT3_set2_f	cloning PCS1	CTGAGGATCCCGCCCGCCTAAAC
QCAT3_set1_r	cloning PCS1	CTAGAAGCTTTCATTCCAGAACGTG

Table S3: Composition of defined M9 for expression of MBP-PCS in *E. coli* AT713.

	final concentration
M9 salts	1X
glucose	0.2 %
thiamine	0.05 mg/ml
D-biotin	0.004 mg/ml
amino acids, each	0.071 mg/ml
$^{13}C_6$, $^{15}N_4$ - Arg $^{13}C_6$, $^{15}N_2$ - Lys	0.071 mg/ml
$CaCl_2$	100 µM
$MgSO_4$	1 mM
$CaCl_2$	20 µM
$MnCl_2$	5 µM
$ZnSO_4$	5 µM
$CoCl_2$	1 µM
$CuCl_2$	0.7 µM
Na_2MoO_4	2 µM
$MgSO_4$	35 µM
H_3BO_3	15 µM
$FeSO_4$	5 µM

Table S4: Raw data IDs used for calculating the mean stoichiometry of each protein.

Found in a separate xls file**.**

Table S5: List of peptides of the proteins of interest used for the construction of PCS1 and PCS2. Three peptides were chosen from the total number of unique peptides detected for each protein (unique peptides include miscleaved forms). Coverage ranged from 14% (SpaR) and 98% (InvG) but was below 50% for all export apparatus proteins. Peptides for PCS are shown in bold, contextual flanking sequences to improve digestion are in regular format. Asterisks indicates position that can cause modification or missed-cleavage.

Protein	PCS1			PCS2		
	Unique peptides detected	Coverage	Peptides	Unique peptides detected	Coverage	Replacement peptides for PCS2
InvA	27	37.17%	ADLK **AGIIDADAAR** E TLAR **NVNEYFGIQETK** HMLD DLDK **VSTETVPLILLVPK** SR	34	45.92%	TVQR **ISEVLQR** LLSE
InvG	34	62.17%	GYTR **DANTDTVQSIPFLGK** LPLI GNIK **IVAYPDTNSLLVK** GTAE PHGK **SLLVGGYTR** DANT	**44**	89.34%	IGDK **LGVSLNQSSISTLDGSR** FIAA
PrgH	26	45.55%	YIVR **LLNSSLNGCEFPLLTGR** TLFV GR **SFQYGAEGYIK** MSPG TWGGR **YVQFAIELK** DDWL	**26**	57.51%	AR **QFVDSYYR** TWGG EK **TITSPGPYIVR** LLNS
PrgJ	7	73.53%	ITNR **IEDPNLVTDPK** ELAI LDDR **LLQAFSGSAIATAVDK** QTIT AR **SIATIVPENAVIGQAVNIR** SME	**10**	98.04%	VNIR **S*METDIVSLDDR** LLQA
PrgK	16	57.71%	AR **LYSAIEQR LEQS** FLK **NSFADVDYDNISVVLSER SDAQ** RPPK **PVHLSALAVYER** GSPL	**16**	62.85%	LSER **SDAQLQAPGTPVK** RNSF
SpaP	7	23.56%	YSD*R **ELVQFFENAQLK** AA SLSK **HVDEGLDGY*RDYLIK** YSD AQL*K ***RQYGEETETV*K*R** D AA	**10**	29.78%	DK **DEIE*KPSIFALLPAYALSEIK** SAFK
SpaQ	2	21.84%	AAR ***MDDLVFAGNK** ALYI SYGR **QVIFLALAK** G A	**4**	22.99%	
SpaR	1	2.65%	FYER **GATHVLE**	**3**	14.02%	LLSR **FAPQ*MNAFAISLTVK** SGIA NVLR **LSFQATGLSSWFYER** GATH
SpaS	3	9.24%	NAGK **DVIQPQENEVR** H A R **EVH*MEILSEQVK** AA YAEK **VGVPVIVDIK** LA	4	12.89%	THRR **YDLVSLEEIDEVLR** LLVW EVLR **LLVWLEEVENAGK** DVIQ

Table S6: Percentage of intensity of peptides of peptide concatenated standard incorporated with light arginine or lysine when grown in M9 medium supplemented only with heavy arginine/lysine.

Protein	Peptide	Intensity percentage of light Arg and Lys incorporated into the PCS
PrgK	NSFADVDYDNISVVLSER	below detection limit
	SDAQLQAPGTPVK	1.78
	SDAQLQAPGTPVKR	below detection limit
PrgJ	LLQAFSGSAIATAVDK	0.34
	LLQAFSGSAIATAVDKQTITNR	below detection limit
	SMETDIVSLDDR	below detection limit
PrgH	LLNSSLNGCEFPLLTGR	below detection limit
	QFVDSYYR	below detection limit
	TITSPGPYIVR	below detection limit
SpaS	LLVWLEEVENAGK	1.9
	RYDLVSLEEIDEVLR	below detection limit
	VGVPVIVDIK	0.1
	VGVPVIVDIKLAR	below detection limit
	YDLVSLEEIDEVLR	below detection limit
SpaR	FAPQMNAFAISLTVK	below detection limit
	LSFQATGLSSWFYER	below detection limit
SpaQ	MDDLVFAGNK	1.49
	QVIFLALAK	1.49
SpaP	DEIEKPSIFALLPAYALSEIK	below detection limit
	ELVQFFENAQLK	1.15
	HVDEGLDGYR	below detection limit
	HVDEGLDGYRDYLIK	below detection limit
	YSDRELVQFFENAQLK	below detection limit
InvA	ISEVLQR	below detection limit
	NVNEYFGIQETK	1.44
	VSTETVPLILLVPK	1.51
	VSTETVPLILLVPKSR	below detection limit
InvG	IVAYPDTNSLLVK	1.53
	LGVSLNQSSISTLDGSR	below detection limit
	SLLVGGYTR	below detection limit