Supplementary Information.

Protein Identification by liquid chromatography tandem mass spectrometry analysis.

Protein spots were manually excised and stored at −80°C until digestion. Gel plugs were washed twice with water and 50% acetonitrile. The cysteine residues were reduced by adding 50 µL of dithiothreitol (20 mmol L^{-1}; Sigma-Aldrich, Shnelldorf, Germany) at 57°C and alkylated by 50 µL of iodoacetamide (100mmol L^{-1}; Sigma-Aldrich) at room temperature. Gel slices were washed three times for 10 minutes each with 200 µL of 40 mmol L^{-1} ammonium bicarbonate in 50% acetonitrile, dehydrated with 100% acetonitrile, and then rehydrated with 20 µL of a 10 mg L^{-1} trypsin (Sigma-Aldrich) solution in 50 mmol L^{-1} ammonium bicarbonate. Trypsin digestion was carried out at 37°C for 12 h. Samples were diluted five folds with 0.1% formic acid (Sigma Aldrich) and 3% acetonitrile and analyzed by nanoLC-MS on a Proxeon EASY-nLCII (Thermo Fisher Scientific, Milan, Italy) interfaced with a maXis HD UHRTOF mass spectrometer (Bruker Daltonik, Bremen, Germany). Two µL of the sample were injected and pre-concentrated on a C18-A1 EASY-ColumnTM (2 cm, 100 mm I.D., 5 mm p.s., Thermo Fisher Scientific) for 1 minute at a flow rate of 10 mL min^{-1} . Trapped peptides were subsequently separated using a C18-Acclaim PepMap (25 cm, 75 mm I.D., 5 mm p.s., Thermo Fisher Scientific). The following operating conditions were applied: flow rate = 0.3 mL min^{-1}; T= 20 °C; eluents: A= 0.1% formic acid in water, B= 0.1% formic acid in acetonitrile; gradient: 2 to 80% B in 60 minutes.

MS/MS data were processed by FlexAnalysis and analyzed using MASCOT MS/ MS ion search engine (http://www.matrixscience.com), searching on the UniProtKB/Swiss-Prot protein knowledgebase with taxonomical restriction for *Salmonella Typhimurium*, the maximal tolerance for peptide masses was 50 ppm and for MS/MS data was 0.3 Da. One miss-cleavage for tryptic peptides was allowed and the variable modification accepted were oxidation of methionines and carbamidomethylation of cysteine.

Supplementary Table S1. Plasmids and oligonucleotides used in this study.

Plasmid	Main features	References
pKD46	Lambda Red recombinase functions	Datsenko and Wanner, 2000
pKD4	Kanamycin resistance cassette template	Datsenko and Wanner, 2000
pKD3	Chloramphenicol resistance cassette template	Datsenko and Wanner, 2000
pCP20	FLP recombinase function	Datsenko and Wanner, 2000
pMC1403	Cloning vector for □-galactosidase activity screening	Casadaban et al., 1980

Oligonucleotide	Sequence	Description
K1	CAGTCATAGCCGAATAGCCT	Internal Kan cassette,reverse
K5	TTTCGTCTCAGCCAATCCCT	Internal Cam cassette, reverse
oli254	ATGAATTCCTGATGGTTTTATCGTAAGT	Cloning of flhDC promoter in pMC1403, forward
oli255	GCGGATCCATCCAGAATAACCAACTTTATT	Cloning of flhDC promoter in pMC1403, reverse
oli256	ATGAATTCGTGAGCAATTATCGATCCC	Cloning of flgM promoter in pMC1403, forward
oli257	GCGGATCCACGATAAACAGCCCTGCG	Cloning of flgM promoter in pMC1403, reverse
oli258	ATGAATTCAGCCGGTACAGCTTACGAT	Cloning of fliA promoter in pMC1403, forward
oli259	GCGGATCCATTTATTTATCCTCATCGAGG	Cloning of fliA promoter in pMC1403, reverse
oli260	ATGAATTCCTGTCAACAACTGGTCTAA	Cloning of fliC promoter in pMC1403, forward
oli261	GCGGATCCACGATCTTTTCCTTATCAATT	Cloning of fliC promoter in pMC1403, reverse
oli272	CAGGTCAGGCGATTGCTAACCGTTTTACCGCGAACATCAATGTAGGCTGGAGCTGCTTCG	Forward for fliC mutant construction
oli273	ATCTTCGATACGGCTACGGGCAGAAGTCAGGTTGTTTACGCATATGAATATCCTCCTTAG	Reverse for fliC mutant construction
oli274	GTCAACAACTGGTCTAACGG	Upstream fliC region, for mutant check
oli275	TCAGGCGATTGCTAACCGTTTCACCGCGAACATCAAAGGTTGTAGGCTGGAGCTGCTTCG	Forward for fljB mutant construction
oli276	CGAGACATGTTGGAAACTTCGGTCGCGTAGTCGGAATCTTCATATGAATATCCTCCTTAG	Reverse for fljB mutant construction
oli277	CTAGTAAGCGCATTACGCTG	Upstream fljB region, for mutant check

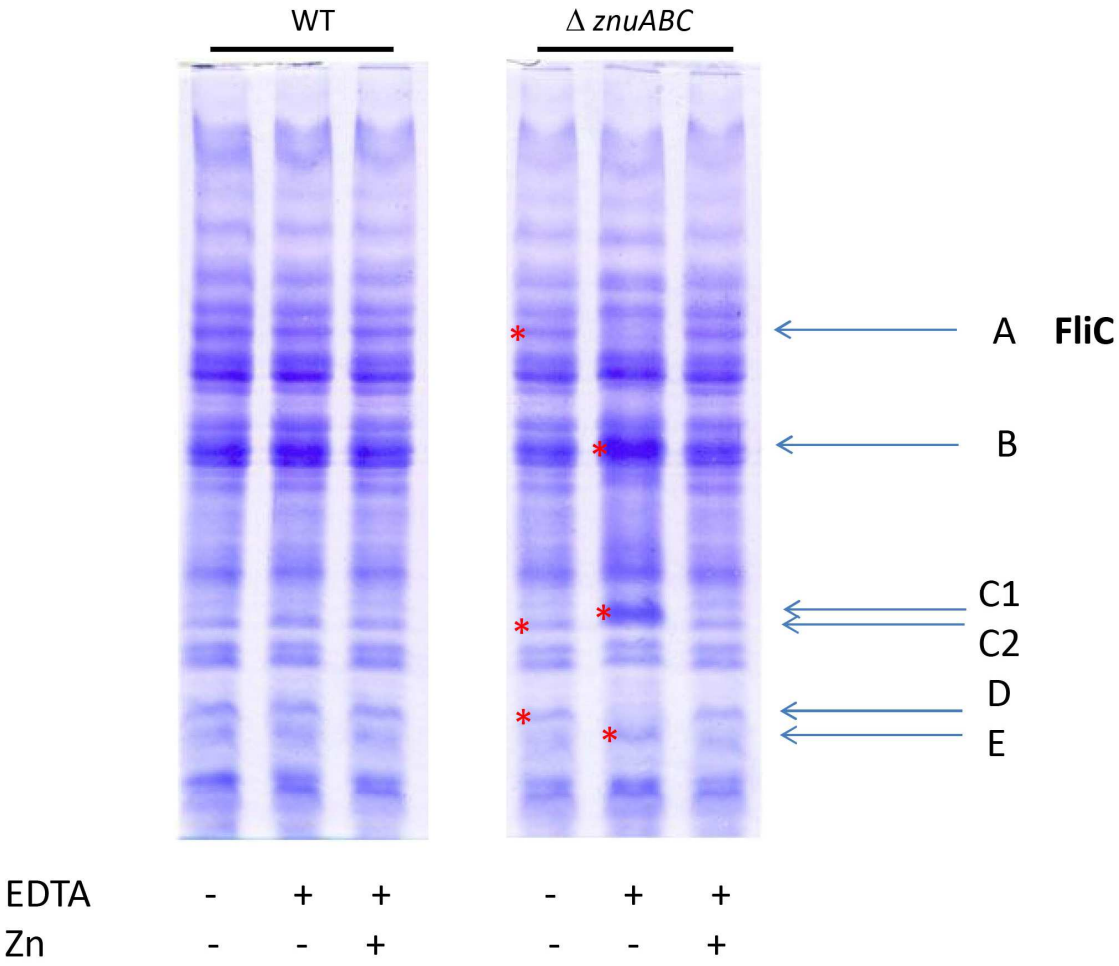

Figure S1: SDS-PAGE analysis of proteins extracted from the wild type and the *znuABC* mutant strains grown in LB medium supplemented or nor with EDTA or EDTA and zinc. Whereas the protein pattern showed by the wild type strain is very similar in all the conditions tested, the profile of the proteins from the *znuABC* mutant strain reveals remarkable changes in presence of EDTA that may be reversed by the addition of zinc. The protein bands indicated by arrows and red stars were excised from the gel and analyzed by Mass spectrometry. The band corresponding to FliC is highlighted.

Supplementary Table S2. Differentially expressed proteins in znuABC mutant strain. Slices A, C2 and D are from LB conditions and are down-regulated in EDTA supplemented medium; slices B, C1 and E are over-expressed in EDTA supplemented medium

spot #	Accession number[a]	Protein name	Theoretical molecular weight	Theoretical isoelectric point	Protein Identification Score[b]	Sequence coverage	Peptide sequence	Precursor	Ion score[c]	Precursor m/z	Error ppm
Gel Slice A	gi 120316	Flagellin	51386	4.99	228	19	326-337	K. VGDDYYSATQNK.D	33	1359.594	0.0120
			51386	4.99	228		391-409	K. AQPDLAEAAATTTENPLQK.I	42	1967.980	0.0168
			51386	4.99	228		431-449	R. FNSAITNLGNTVNNLTSAR.S	0	2006.018	0.0184
			51386	4.99	228		450-466	R.	23	1974.859	0.0163
			51386	4.99	228		467-493	R.	52	2859.593	0.0271
			51386	4.97	228		467-493	R.	130	2859.593	0.0272
	gi	Flagellin	51848	4.65	205	16	330-341	K. VGDDYYSATQNK.D	33	1359.594	0.0120
			51848	4.65	205		388-413	K.	20	2765.362	0.0240
			51848	4.65	205		454-470	R.	23	1974.859	0.0163
			51848	4.65	205		471-497	R.	130	2859.593	0.0272
	gi	ATP synthase	50309	4.9	191	24	54-64	R.TIAMGSSDGLR.R	19	1106.539	0.0090
			50309	4.9	191		71-82	K. DLEHPIEVPVGK.A	26	1331.709	0.0084
			50309	4.9	191		88-99	IMNVLGEPVDMK	24	1344.678	0.0107
			50309	4.9	191		157-165	TVNMMELIR	20	1105.562	0.0093
			50309	4.9	191		220-230	VALTGLTMAEK	42	1132.616	0.0093
			50309	4.9	191		262-282	MPSAVGYQPTLAEEMGVLQER	20	2305.108	0.0222
			50309	4.9	191		360-367	GVQSILQR	19	899.519	0.0062
			50309	4.9	191		430-456	GIMEGEYDHLPEQAFYMVGSIDEAVEK	21	3056.378	0.0275
	gi	phase 1 flagellin	52496	4.66	183	12	394-412	AQPDLAEAAATTTENPLQK	30	1967.980	0.0168
			52496	4.66	183		453-469	SRIEDSDYATEVSNMSR	23	1974.859	0.0163
			52496	4.66	183		470-496	AQILQQAGTSVLAQANQVPQNVSLLR	130	2859.593	0.0272
	gi	Flagellin	52081	4.85	153	8	458-474	SRIEDSDYATEVSNMSR	23	1974.859	0.0163
			52081	4.85	153		475-501	AQILQQAGTSVLAQANQVPQNVSLLR	130	2859.593	0.0272

[a] gi number in NCBInr database. [b] Mascot score is given as $S = -10 \times \log(P)$, where P is the probability that the observed match is a random event. [c] Ion score is given as $S = -10 \times \log(P)$, where P is the probability of the peptide being a random match to its corresponding MS/MS spectrum

Supplementary Table S2. Continued.

spot #	Accession number[a]	Protein name	Theoretical molecular weight	Theoretical isoelectric point	Protein Identification Score[b]	Sequence coverage	Peptide sequence	Precursor	Ion score[c]	Precursor m/z	Error ppm
Gel Slice A	gi 112819037	ATP synthase beta subunit	28127	5.35	125	25	36-74	DLEHPIEVPVGK	18	1330.725	0.0084
			28127	5.35	125		53-64	IMNVLGEPVDMK	24	1344.678	0.0107
			28127	5.35	125		122-130	TVNMMELIR	20	1105.562	0.0093
			28127	5.35	125		185-195	VALTGLTMAEK	42	1132.616	0.0093
			28127	5.35	125		227-247	MPSAVGYQPTLAEEMGVLQER	20	2305.108	0.0222
	gi 49363	Rho Factor	47007	6.75	63	4	259-269	DVIILLDSITR	44	1256.734	0.0109
			47007	6.75	63		354-362	VFPAIDYNR	19	1093.556	0.0088
	gi 2495191	Outher membrane protein tolC	53750	5.42	58	4	244-251	NLSLLQAR	20	913.535	0.0058
			53750	5.42	58		389-404	TIVDVLDATTTLYDAK	38	1737.904	0.0158
	gi 16759834	seryl-tRNA synthetase	48835	5.39	45	5	39-51	VLQVNTENLQAER	21	1512.790	0.0129
			48835	5.39	45		248-259	DEIIDEDQLPIK	23	1426.719	0.0129
Gel Slice B	gi 62179873	gapA gene product	36101	6.33	141	20	128-135	DVDNNMNL	12	950.453	1.0809
			36101	6.33	141		136-163	GANFDKYEGQDIVSNASCTTNCLAPLAK	33	3043.402	0.0365
			36101	6.33	141		253-260	AATYEQIK	10	922.476	0.0083
			36101	6.33	141		300-310	AGIALNDNFVK	30	1160.619	0.0135
			36101	6.33	141		311-324	LVSWYDNETGYSNK	56	1674.753	0.0219
	gi 16759000	transaldolase B	35320	5.09	95	14	10 25	QFTTVVADTGDIAAMK	18	1666.824	0.0196
			35320	5.09	95		93-100	ISTEVDAR	22	889.451	0.0043
			35320	5.09	95		229-241	NVGEILELAGCDR	48	1444.698	0.0178
			35320	5.09	95		242-250	LTIAPALLK	7	938.616	0.0088
	gi 758341	outer membran protein ompA	37680	5.6	80	7	118-124	LGGMVWR	14	833.422	0.0056
			37680	5.6	80		303-321	GMGESNPVTGNTCDNVKPR	67	2031.910	0.0231
	gi 16764916	outer membran porin precursor	39655	4.66	60	14	63-82	GETQINDQLTGFGQWEYEFK	1	2389.086	0.0298
			39655	4.66	60		301-324	NLGTYGDQDLVEYIDVGATYYFNK	18	2757.281	0.0353
			39655	4.66	60		325-333	NMSTFVDYK	41	1119.491	0.0124

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Supplementary Table S2. Continued.

spot #	Accession number[a]	Protein name	Theoretical molecular weight	Theoretical isoelectric point	Protein Identification Score[b]	Sequence coverage	Peptide sequence	Precursor	Ion score[c]	Precursor m/z	Error ppm
Gel Slice B	gi 16762600	ADP-L-glycero-D-manno-heptose-6-epimerase	34941	4.91	42	11	23-34	GITDILVVDNLK	7	1298.745	0.0157
			34941	4.91	42		108-123	EIPFLYASSAATYGGR	19	1701.836	0.0206
Gel Slice C1	gi 16760462	glutathionine S-transferase	22563	6.09	46	17	37-49	LENGDDYLAVNPK	37	1446.699	0.0170
			22563	6.09	46		151-163	FTIADAYLFTVLR	7	1528.829	0.0251
			22563	6.09	46		188-196	RPTVAAALK	2	925.571	0.0124
	gi 808038	Mn-superoxide dismutase	22952	6.05	100	27	2 30	SYTLPSLPYAYDALEPHFDKQTMEIHHTK	7	3431.650	0.0513
			22952	6.05	100		106-119	DFGSVDNFKAEFEK	48	1631.747	0.0251
			22952	6.05	100		188-201	EFWNVVNWDEAAAR	45	1705.785	0.0266
	gi 16759380	peroxidase	22417	5.24	77	18	68-86	GVEVVGVSFDSEFVHNAWR	21	2133.028	0.0321
			22417	5.24	77		139-149	HQVVNDLPLGR	37	1246.678	0.0188
			22417	5.24	77		150-156	NIDEMLR	19	889.433	0.0115
	gi 16759997	TrpR binding protein Wrba	20824	5.78	68	18	55-79	TQNAPVATPQELADYDAIIFGTPTR	40	2688.340	0.0398
			20824	5.78	68		173-183	QPSQEELSIAR	28	1256.636	0.0202
Gel Slice C2	gi 16759380	peroxidase	22417	5.24	44	15	157-177	MVDALQFHEEHGDVCPAQWEK	26	2525.110	0.0405
			22417	5.24	44		192-200	YLAENISSL	18	1008.513	0.0177

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Supplementary Table S2. Continued.

spot #	Accession number[a]	Protein name	Theoretical molecular weight	Theoretical isoelectric point	Protein Identification Score[b]	Sequence coverage	Peptide sequence	Precursor	Ion score[c]	Precursor m/z	Error ppm
Gel Slice D											
	gi 15803854	rpsG gene product	17593	10.3	70	10	37-53	STAESIVYSALETLAQR	70	1837.942	0.0127
Gel Slice E	gi 16762840	bacterioferritin	18370	4.64	123	41	62-76	ILFLEGIPNLQDLGK	70	1668.945	0.0278
			18370	4.64	123		77-88	LGIGEDVEEMLR	17	1375.665	0.0225
			18370	4.64	123		118-143	DMMIEILADEEGHIDWIETELDLIAK	2	3041.461	0.0318
			18370	4.64	123		144-155	LGMQNYLQSQIK	34	1421.734	0.0226
	gi 15803854	rpsG gene product	17593	10.3	85	21	37-53	STAESIVYSALETLAQR	51	1837.942	0.0298
			17593	10.3	85		37-53	STAESIVYSALETLAQR	10	1837.942	0.0299
			17593	10.3	85		80-95	VGGSTYQVPVEVRPVR	33	1741.948	0.0268
	gi 16759236	outer membrane lipoprotein	14522	9.19	60	10	51-64	IYTNAEDLVGKPFR	60	1621.846	0.0236
	gi 16762303	50s ribosomal protein L10	17846	9.04	47	6	21-31	GALSAVVADSR	47	1044.556	0.0153
	gi 15803830	rpsE gene product	17592	10.11	63	22	94-112	VGGSTYQVPVEVRPVR	44	1891.928	0.0284
			17592	10.11	63		94-112	VFMQPASEGTGIIAGGAMR	12	1907.923	0.0302
			17592	10.11	63		149-156	ATIDGLENMNSPEMVAAK	20	1889.886	0.0294

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