

Supplemental Table 2. Proteome analysis in *N. meningitidis* infected to HBMEC

Accession No.	Description	Exp.1		Exp.2		Exp.3	
		Mutant / WT	Average	Mutant / WT	Average	Mutant / WT	Average
15676484	copper ABC transporter ATP-binding protein [<i>Neisseria meningitidis</i> MC58]	0.738	1.169	1.761	1.473	/	/
15676734	cold-shock domain-contain protein [<i>Neisseria meningitidis</i> MC58]	0.370	0.586	0.589	0.493	/	/
15677308	hypothetical protein NMB1452 [<i>Neisseria meningitidis</i> MC58]	0.821	1.301	1.680	1.406	0.665	1.397
161353785	riboflavin synthase subunit alpha [<i>Neisseria meningitidis</i> MC58]	/	/	0.759	0.635	0.348	0.731
218767155	30S ribosomal protein S14 [<i>Neisseria meningitidis</i> Z2491]	0.765	1.213	/	/	0.627	1.318
254672983	putative exported protein [<i>Neisseria meningitidis</i> alpha275]	0.340	0.538	0.700	0.586	0.224	0.472
254673507	3-isopropylmalate dehydratase, small subunit [<i>Neisseria meningitidis</i> α 275]	0.414	0.656	0.586	0.49	0.278	0.585
254674245	putative thioredoxin [<i>Neisseria meningitidis</i> alpha275]	0.341	0.540	/	/	0.341	0.540
316985219	hypothetical protein NMH_1017 [<i>Neisseria meningitidis</i> H44/76]	1.195	1.894	2.939	2.459	1.045	2.196
254671740	thioredoxin [<i>Neisseria meningitidis</i> alpha153]			0.507	0.424	0.193	0.406
254672281	outer membrane lipoprotein carrier protein LolA [<i>Neisseria meningitidis</i> α 275]	/	/	0.715	0.598	0.337	0.707
254673840	ADP-ribose pyrophosphatase [<i>Neisseria meningitidis</i> α 275]	/	/	0.612	0.512	0.271	0.569
254674279	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase [<i>Neisseria meningitidis</i> α 275]	/	/	0.391	0.327	0.319	0.669
296061833	ABC transporter, periplasmic substrate-binding protein [<i>Neisseria</i>	/	/	0.702	0.587	0.351	0.783

	<i>meningitidis</i>]						
316985219	hypothetical protein NMH_1017 [<i>Neisseria meningitidis</i> H44/76]	1.195	1.894	2.939	2.459	1.045	2.196
352289494	CtrB [<i>Neisseria meningitidis</i>]	1.032	1.636	1.715	1.435	0.954	2.006
385852822	antibiotic biosynthesis monooxygenase family protein [<i>Neisseria meningitidis</i> H44/76]	0.410	0.649	0.504	0.422	0.281	0.591
385853257	hypothetical protein NMBH4476_1113 [<i>Neisseria meningitidis</i> H44/76]	0.411	0.651	0.695	0.582		
385853541	DNA polymerase III subunit delta' [<i>Neisseria meningitidis</i> H44/76]	0.887	1.406			0.607	1.276
385854001	50S ribosomal protein L31 [<i>Neisseria meningitidis</i> H44/76]	0.381	0.604	0.542	0.453		
385854176	hypothetical protein NMBH4476_2082 [<i>Neisseria meningitidis</i> H44/76]	0.377	0.597	0.606	0.507	0.130	0.274
389606666	hypothetical protein NMALPHA522_2037 [<i>Neisseria meningitidis</i> α 522]	0.781	1.237	1.892	1.583		
488143866	adhesin [<i>Neisseria meningitidis</i>]	0.360	0.571	0.639	0.535	0.268	0.562
488148010	hypothetical protein [<i>Neisseria meningitidis</i>]	0.376	0.596			0.269	0.566
488149008	glyceraldehyde-3-phosphate dehydrogenase [<i>Neisseria meningitidis</i>]	0.802	1.271	2.135	1.786	0.617	1.297
488162207	50S ribosomal protein L3 [<i>Neisseria meningitidis</i>]	0.391	0.62	0.769	0.644	0.684	1.438
488164052	ATP-dependent DNA helicase HrpA [<i>Neisseria meningitidis</i>]	0.764	1.211	1.774	1.484		
488165487	beta-phosphoglucomutase [<i>Neisseria meningitidis</i>]	0.341	0.54			0.345	0.726
488169334	hypothetical protein [<i>Neisseria meningitidis</i>]	0.786	1.246			0.732	1.538
488177979	neisseria PilC beta-propeller domain protein [<i>Neisseria meningitidis</i>]	0.410	0.65			0.347	0.729
488188694	hypothetical protein [<i>Neisseria meningitidis</i>]	0.758	1.201	1.642	1.374		
498490231	putative 3-deoxy-manno-octulosonate cytidyltransferase [<i>Neisseria</i>	0.843	1.336	1.772	1.483	#	#

	<i>meningitidis</i>]						
500123036	endo-1,4-D-glucanase [<i>Neisseria meningitidis</i>]	0.371	0.588	0.519	0.435	0.294	0.618
501178742	N5-glutamine S-adenosyl-L-methionine-dependent methyltransferase [<i>Neisseria meningitidis</i>]	0.821	1.302	1.686	1.411	0.772	1.623
501178803	glutamate racemase [<i>Neisseria meningitidis</i>]	0.781	1.238	1.971	1.649		
515768390	hypothetical protein [<i>Neisseria meningitidis</i>]	0.211	0.334	0.681	0.57	0.177	0.371
545064626	acyl-CoA dehydrogenase, N-terminal domain protein [<i>Neisseria meningitidis</i>]	0.353	0.559			0.318	0.667
316985685	ribosomal L18p/L5e family protein [<i>Neisseria meningitidis</i> H44/76]			0.646	1.357	1.513	1.266
332639892	Chain A, The Crystal Structure Of Thioredoxin-Related Protein From <i>Neisseria meningitidis</i> Serogroup B			0.633	0.53	0.217	0.456
374074225	Chain D, Fic Protein From <i>Neisseria meningitidis</i> Mutant Delta8 In Complex With AMP-PNP			0.720	0.602	0.325	0.684
385852525	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase [<i>Neisseria meningitidis</i> H44/76]			0.746	0.624	0.333	0.701
385852535	hypothetical protein NMBH4476_0340 [<i>Neisseria meningitidis</i> H44/76]			0.589	0.439	0.333	0.701
385852828	cytosol aminopeptidase [<i>Neisseria meningitidis</i> H44/76]			0.758	0.634	0.342	0.719
385852892	ribonuclease PH [<i>Neisseria meningitidis</i> H44/76]			0.668	0.559	0.354	0.744
385852936	hypothetical protein NMBH4476_0776 [<i>Neisseria meningitidis</i> H44/76]			0.738	0.617	0.299	0.628
385852989	glucose-6-phosphate isomerase [<i>Neisseria meningitidis</i> H44/76]			1.693	1.416	0.317	0.666
385853537	glutaredoxin [<i>Neisseria meningitidis</i> H44/76]			0.444	0.372	0.326	0.686

385853547	cysteine synthase A [<i>Neisseria meningitidis</i> H44/76]			0.808	0.676	0.355	0.747
385853647	endoribonuclease L-PSP [<i>Neisseria meningitidis</i> H44/76]			0.804	0.672	0.334	0.702
385853669	inorganic pyrophosphatase [<i>Neisseria meningitidis</i> H44/76]			0.618	0.517	0.352	0.74
385853871	shikimate kinase [<i>Neisseria meningitidis</i> H44/76]			0.739	0.618	0.290	0.609
385854073	3-demethylubiquinone-9 3-methyltransferase [<i>Neisseria meningitidis</i> H44/76]			0.710	0.594	0.254	0.534
488147722	hypothetical protein, partial [<i>Neisseria meningitidis</i>]			0.682	0.571	0.332	0.674
488152897	3-ketoacyl-ACP reductase [<i>Neisseria meningitidis</i>]			0.745	0.624	0.346	0.702
488155421	aromatic amino acid aminotransferase [<i>Neisseria meningitidis</i>]			0.783	0.655	0.297	0.624
488155967	phosphoglycolate phosphatase, bacterial, partial [<i>Neisseria meningitidis</i>]			0.672	0.562	0.280	0.589
488164122	ABC transporter substrate-binding protein [<i>Neisseria meningitidis</i>]			0.749	0.627	0.277	0.582
488181049	glyceraldehyde-3-phosphate dehydrogenase, type I [<i>Neisseria meningitidis</i>]			0.688	0.576	0.276	0.579
488186253	hypothetical protein, partial [<i>Neisseria meningitidis</i>]			0.710	0.594	0.266	0.56
488190078	3-deoxy-D-manno-octulosonate 8-phosphate phosphatase, YrbI family protein, partial [<i>Neisseria meningitidis</i>]			0.727	0.608	0.281	0.591
504393427	ATP-dependent metalloprotease [<i>Neisseria meningitidis</i>]			1.668	1.396	0.608	1.278

Protein samples were prepared as follows: bacteria infected to HBMEC (see Materials and Methods) were suspended in 70 μ l of 100 mM $\text{Na}_2\text{B}_4\text{O}_7$ (pH 7.5). Ten μ l of 20 mM Tris (2-chloroethyl)phosphate (TCEP) and 10 μ l of 10% SDS were added, and then the

bacterial suspension was incubated at 95 °C for 30 min. The sample was lightly sonicated for 5 sec and centrifuged at 16,000 x g at 4 °C for 5 min. The resultant supernatant (~100 µl) was mixed with 900 µl of 100 mM Na₂B₄O₇ (pH 7.5), and the protein concentration was determined with a BCA Protein Assay Kit (Thermo), with bovine serum albumin as the standard. A solution corresponding to 100 µg of protein was mixed with triethylammonium bicarbonate (TEAB) and TCEP, at final concentrations of 45 mM and 10 mM, respectively. The solution was incubated at 55 °C for 1 hr and then cooled to room temperature. A 15 µl aliquot of 375 mM iodoacetoamide was added to the solution, which was incubated at room temperature for 30 min under dark conditions. The protein was precipitated with chilled acetone. The pellet was dried, suspended in 100 µl of 100 mM TEAB, and digested with trypsin at 37 °C overnight. The resultant protein samples were labeled with a TMTduplex Isobaric Mass Tagging Kit (Thermo), according to the manufacturer's protocol. The samples were subjected to an LC-MS/MS analysis. The results were analyzed by Proteome Discovery (Ver. 1.4) with the MASCOT search engine (Ver. 2.4.1), referring to the NCBI_Neisseria meningitidis (entry number: 453988), NCBI_Neisseria meningitidis_H44/76

(entry number: 6401) and NCBI_Neisseria meningitidis_MC58 (entry number: 4914) databases. The referring databases for the results are shown in the Table with square brackets. The cut-offs for the false discovery rate and the peptide rank for all analyzed data were $<5\%$ and 1, respectively. Since the protein expression ratios in the 3 independent experiments varied, the ratio value was divided by the average in each assay for normalization (shown in gray columns). Proteins with expression ratios of mutant (M) per wild type (W) strains (M/W) between 1.25 and 0.75 were eliminated, and proteins found in 2 out of 3 total independent experiments were chosen as reproducible results and shown in the table.