Text S1

MS-H typing of *Salmonella* flagella. Reference strains were cultured overnight without motility induction. Colonies from one plate were harvested on a 10 μl inoculation loop and transferred to a 1.5 ml centrifuge tubes (Eppendorf) containing 1 ml de-ionized water (dH_2O) and 2 mg lysozyme (Sigma). The suspension was gently mixed by a pipette tip and left at room temperature for 10 minutes. After this, the mixture was vigorously vortexed for 20 seconds three times, with a break of 2 minutes between votexing. The mixture was then centrifuged for 20 minutes at 16,000 x g and the supernatant collected using a 1 ml syringe. It was then passed through a 13 mm diameter filter with 0.22 μm pore size and low protein binding capability (PALL). The filter was washed with 3 ml of dH_2O and filled with a solution containing 100 ng/μl sequence grade trypsin (Promega) after the residual water was expelled using a new syringe. The on-filter digestion was performed at 37°C for 2 hours and the digest collected by flushing the filter with 0.6 ml dH_2O. The tryptic digest from the filter was expelled completely using the syringe, and 25 μl of HPLC 2X buffer A (0.2% formic acid, 2% Acetonitrile) was added to 25 μl digest. Ten μl of the sample, containing 1/120 of the total tryptic digest, was loaded onto a 0.1 x 3 mm self-packed C18 pre-column for 8 minutes and the pre-column automatically switched to a self-packed C18 nano-LC column. Nano-LC (Proxeon) separation was run at 250 nl/min with a 35 minute acetonitrile gradient from 5-30% followed by a 20 minute flush with 95% acetonitrile. Columns were equilibrated for 10 minutes with buffer A before loading the sample. Mass spectrometry data were collected from LTQ-Orbitrap XL system (ThermoFisher)) with a data-dependent acquisition method for peptide ion scanning and fragmentation. For multiple samples runs together in high throughput mode, two blank runs were performed after each sample, the first being called “jigsaw” to wash the nano-column vigorously with several cycles of high concentration acetonitrile, and the second using the same gradient as a true sample run to re-flush and equilibrate the column for the next sample. The mass spectrometry data were searched with Mascot 2.3 (Matrix Science) against a custom *Salmonella* flagella database containing 385 different sequence entries. Search parameters included two missed cleavages of trypsin digestion with no fixed modifications of proteins and possible modifications of methionine oxidation and glutamine and asparagine deamidation.

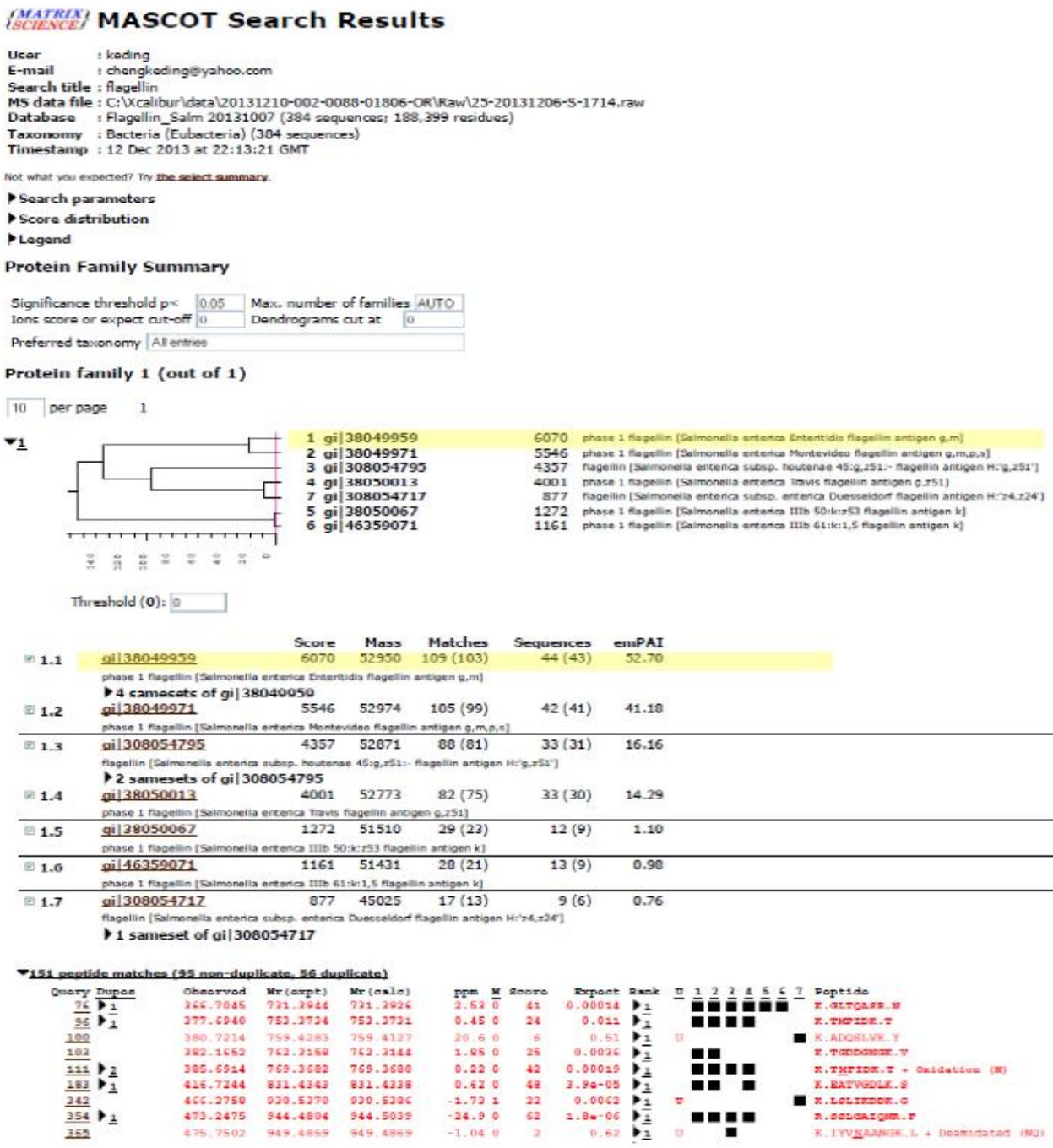

Fig. S1 Snapshot of MS-H identification of monophasic *Salmonella* Enterititis [9,12:g,m:-] flagella. Original MS data of flagella trypsin digest was searched against all 385 known *Salmonella* flagellin sequences. The dendrogram was produced using the sequence data of all hits (only the top seven hits are shown), its units representing "peptide ions score" which is correlated with the confidence of the identified peptides. The vertical red line on the dendrogram shows the peptide ion score cut-off value for grouping identified proteins, which was set to "0" here. Individual rows display the results of each protein search summary by way of accession number, protein score, protein molecular weight (mass), number of matches (redundant) and number of distinct sequences (non-redundant). The numbers outside parentheses in both the "Matches" and "Sequences" columns represent the total count of identified peptides, while the numbers in parentheses denote the number of matches above the significance threshold. "emPAI" denotes the relative quantitation [i.e. ten to the power of (the number of observed peptides divided by the number of observable peptides), minus 1]. The top hit (1.1), showing the highest score and emPAI value, is regarded as the correct flagella type (g,m).

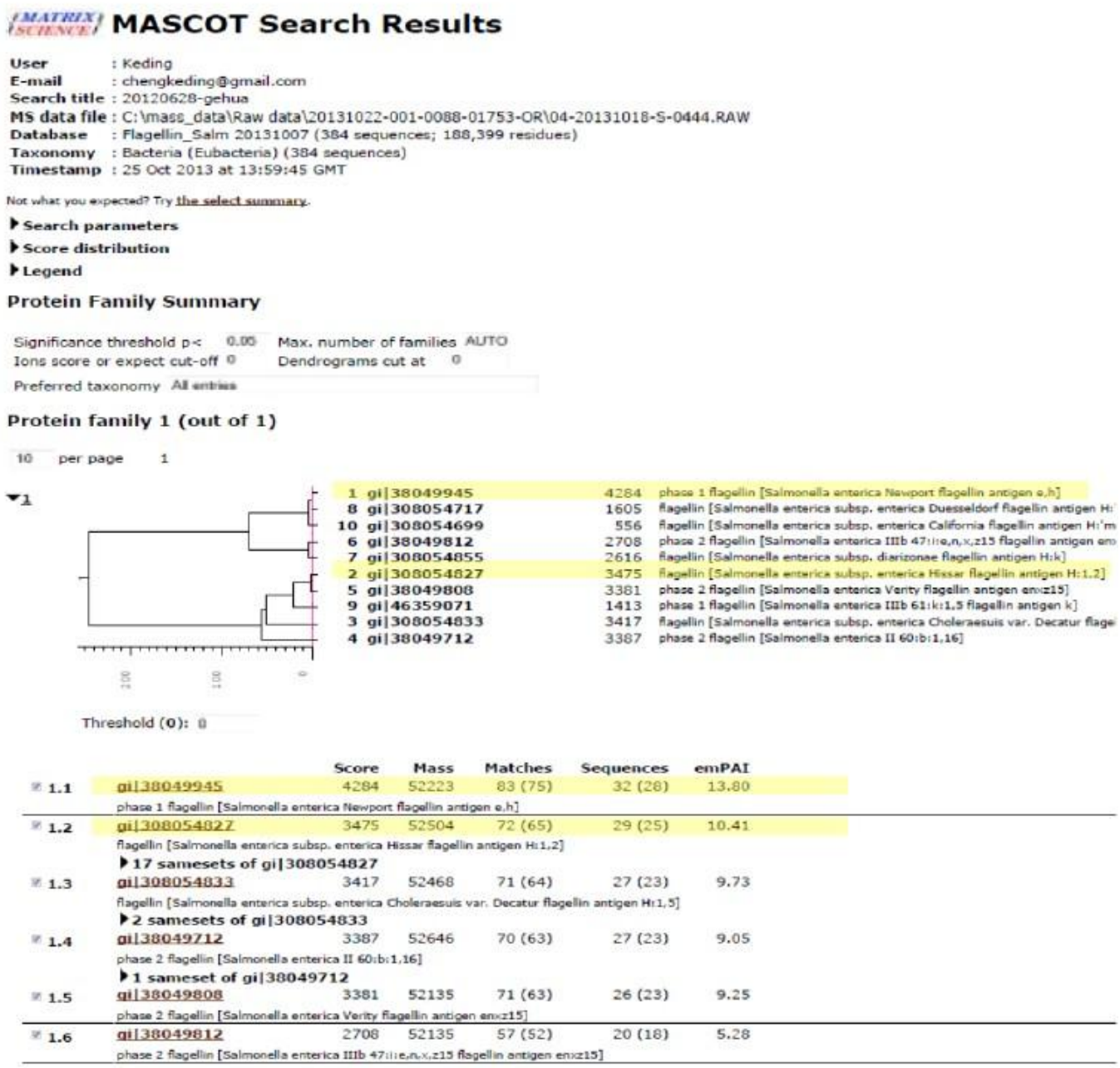

Fig. S2 Snapshot of MS-H identification of diphasic *Salmonella* Newport [6,8:e,h:1,2] flagella. Original MS data of flagella trypsin digest was searched against all 385 known *Salmonella* flagellin sequences. The dendrogram was produced using the sequence data of all hits (only the top 6 hits are shown), its units representing "peptide ions score" which is correlated with the confidence of the identified peptides. The vertical red line on the dendrogram shows the peptide ion score cut-off value for grouping identified proteins, which was set to "0" here. Two distinct groupings, signifying phase 1 and phase 2 antigens, can easily be seen. Individual rows display the results of each protein search summary by way of accession number, protein score, protein molecular weight (mass), number of matches (redundant) and number of distinct sequences (non-redundant). The numbers outside parentheses in both the "Matches" and "Sequences" columns represent the total count of identified peptides, while the numbers in parentheses denote the number of matches above the significance threshold. "emPAI" denotes the relative quantitation [i.e. ten to the power of (the number of observed peptides divided by the number of observable peptides), minus 1]. The top hits (1.1 and 1.2), showing the highest scores and emPAI values, are regarded as the correct flagella types (e,h and 1,2 representing phase 1 and phase 2 antigens, respectively).

TABLE S1 Results of MS-H analyses using different loading amounts of flagellin digest produced from *Salmonella enterica* serovar Newport (6,8:e,h:1,2)

Loading amount[a]	Phase 1 H antigen top hit	Phase 2 H antigen top hit
1/5000	e,h	1,7
1/500	e,h	1,5
1/50	e,h	1,2[b]

[a]Loading amount represents the fraction of the 600 µl flagellin digest used for MS-H.

[b]1,2 denotes the correct H type for the strain under study.

TABLE S2 Results of MS-H analyses using different loading amounts of flagellin digest produced from *Salmonella enterica* Infantis (6,7:r:1,5)

Loading amount[a]	Phase 1 H antigen top hit	Phase 2 H antigen top hit
1/5000	b	1,5
1/500	r,i	1,5
1/50	r[b]	1,5

[a]Loading amount represents the fraction of the 600 µl flagellin digest used for MS-H.

[b]"r" denotes the correct H type for the strain under study.