

Table S14. Summary of regions of significant constraint found in H5N8 (avian host) influenza A genes, using weighted and ranked codon variability values. Reference sequences used are GenBank LC699653.1, LC699654.1, LC699655.1, LC699656.1, LC699657.1, LC699658.1, LC699659.1, LC699660.1, for segments 1–8, respectively. Citation details may be found in S1 Appendix. *Denotes a region only found by excluding a potentially interfering signal. *Z*- and *p*-values in parentheses denote values prior to removal of the next most significant signal. If parenthetical values are absent, then such a signal was removed in an earlier step only.

| Gene | Order found | Refseq nt location | <i>Z</i> | <i>p</i> | Comment |
|--------|-------------|--------------------|----------|----------|--|
| PB2 | 2 | 66–113 | 2.84 | 0.0003 | Packaging-associated(21, 22); conserved RNA structure(18) |
| | 1 | 2229–2288 | 4.22 | <0.0001 | Packaging-associated(4–6, 21, 23, 24); conserved RNA structure(3, 25) |
| PB1 | 1 | 2248–2289 | 2.86 | 0.0009 | Packaging-associated(5, 6, 21, 22); conserved RNA structure(3, 18) |
| PB1-F2 | 1 | 293–334 | 1.93 | <0.0001 | PB1-N92 initiation region (see main text); conserved RNA structure(18) |
| PA | 2 | 584–763 | 2.48 | 0.0006 | Proposed frameshift stimulator (see main text); overlap PA-X(26) |
| | 1 | 2072–2149 | 3.19 | <0.0001 | Packaging-associated(5, 6, 21) – but longer than previously described regions |
| PA-X | 1 | 744–764 | 2.48 | 0.0006 | Overlap PA |
| HA | 2* | 791–877 | 1.94 | 0.0296 | Unclear |
| | 1 | 1646–1687 | (1.80) | (0.2937) | Packaging-associated(8, 9, 27) |
| NP | 2 | 48–68 | 2.59 | 0.0022 | Packaging-associated(3, 28); conserved cRNA structure(18) |
| | 1 | 1446–1517 | 2.75 | 0.0009 | Packaging-associated(28, 29, 31) – but longer than previously described region; conserved RNA structure(3, 31) |
| NA | 2* | 287–571 | 1.98 | 0.0364 | Unclear; conserved cRNA structure(18) |
| | 1 | 1331–1420 | (1.73) | (0.2667) | Packaging-associated(31–33); conserved cRNA structure(18) |
| M1 | 1 | 70–252 | 2.72 | <0.0001 | Packaging-associated(7, 16); M2 splice donor; M42 alternate ORF and m4 splice junction(17); conserved RNA structure(3, 14, 15, 18) |
| M2 | Nil found | | | | |
| NS1 | 1* | 44–178 | 2.02 | 0.0004 | Packaging-associated(11); splice donor; conserved RNA structure(3, 15, 37, 38) |
| | 3* | 467–520; 599–667 | (1.67) | (0.1496) | Overlapping ORFs |
| | 2 | 521–598 | 1.77 | 0.0134 | Splice acceptor; conformationally important region(20); overlapping ORFs |
| NS2 | 1 | 44–52; 525–620 | 2.30 | <0.0001 | Splice donor/acceptor; conformationally important region(20); overlapping ORFs |