

**Table S6. Summary of regions of significant constraint found in H5N8 (avian host) influenza A genes, using weighted and raw (un-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	66–152	1.27	0.0118	Packaging-associated(21, 22); conserved RNA structure(18)
	1	2106–2303	1.54	<0.0001	Packaging-associated(4–6, 21, 23, 24); conserved RNA structure(3, 25)
PB1	1 <sup>†</sup>	2134–2295	0.78 (0.70)	0.0403 (0.1318)	Packaging-associated(5, 6, 21, 22) – note region described extends 5' of previously described regions; conserved RNA structure(3, 18)
PB1-F2	Nil found				
PA	1	584–778	0.86	0.0067	Proposed frameshift stimulator (see main text); overlap PA-X(26)
	2	2015–2161	0.86	0.021	Packaging-associated(5, 6, 21) – but longer than previously described regions; conserved cRNA structure(18)
PA-X	1 <sup>†</sup>	603–779	0.70 (0.60)	0.0217 (0.0776)	Proposed frameshift stimulator (see main text); overlap PA
HA	Nil found				
NP	2 <sup>†</sup>	48–296	0.82 (0.71)	0.0176 (0.0947)	Packaging-associated(28–30) – note less conservation in portion that does not overlap with previously described region; conserved RNA structure(3, 18)
	3 <sup>†</sup>	1158–1268	0.86 (0.79)	0.0299 (0.0571)	Unclear; conserved cRNA structure in one subregion(18)
	1	1359–1541	0.83	0.0007	Packaging-associated(28, 29, 31) – but longer than previously described region; conserved RNA structure(3, 31)
NA	2 <sup>†</sup>	263–502	0.60 (0.41)	0.0311 (0.6983)	Unclear
	3 <sup>†</sup>	689–1270	0.48	0.0016	Conserved RNA structure(3)
	1 <sup>†</sup>	1307–1423	0.56 (0.51)	0.0229 (0.0505)	Packaging-associated(31–33); conserved cRNA structure(18)
M1	1	25–255	0.69	0.0003	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	44–136	0.51	0.0004	Packaging-associated(11); splice donor; conserved RNA structure(3, 15, 37, 38)
	2	467–667	0.60	0.0224	Splice acceptor; conformationally important region(20); overlapping ORFs
NS2	Nil found				