Supplementary Information

Genome-wide Analysis of Influenza Viral RNA and Nucleoprotein Association

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Supplementary Figure Legends

Supplementary Figure S1. NP HITS-CLIP profiles of independent biological replicates for WSN strain. The NP binding profiles of all eight segments are shown for two replicate experiments using the monoclonal HB65 antibody (black and green tracks). The overall Pearson correlation coefficient is 0.798, indicating a highly correlative binding profile. The NP binding profile derived from HITS-CLIP with a distinct anti-NP antibody (Millipore MAB8251) is shown as the red track. The Pearson correlation coefficient between Replicate 1 and the profile generated with the second antibody is 0.9291 for the experiment shown here and 0.7185 for a replicate experiment, which is also deposited in the SRA database (please see **Supplementary Table S3**).

Supplementary Figure S2. RNA-seq tracks for WSN segments from total RNA input. RNA was extracted from purified virions, and tagmentation-based library preparation was performed followed by deep sequencing. Note that 5′ and 3′ ends of all segments are underrepresented as the frequency of Tn5 transposition is decreased towards the termini.

Supplementary Figure S3. Accessibility assay of vRNA by ASO-mediated RNase H digestion. (**A-B**) As shown in Figure 3B, vRNA accessibility assay was performed either on WSN viral lysate containing vRNA segments complexed with NP or naked RNA. DNA ASOs targeting NP-binding sites (ASO #5, #8, and #9) and NP-free regions (ASO #6, #7, and #10) of HA and NS segments were used. Their locations within the segments are indicated underneath the tracks. The presence of ASO #5, #8, and #9 does not result in efficient RNase H-mediated degradation using vRNP substrates, while addition of ASO #6, #7, and #10 result in cleavage of the respective segments. Northern blot analysis for HA and NS segments was carried out to examine accessibility as determined by RNase H digestion. Arrows indicate full-length/non-

degraded segments. Northern blot against PB2 segment is shown as a negative control. Functionality of DNA ASOs in mediating RNase H digestion is demonstrated by using these ASOs in combination with naked purified viral RNA as substrate. (**C**) CIMS are enriched around NP-binding sites. Distribution of all WSN NP-binding sites (HITS-CLIP peaks, blue) and CIMS (red) relative to peak center (set to 0 nt). Sites of statistically significant (FDR < 0.001) CIMS including deletions and substitutions are plotted relative to the peak centers. The majority of CIMS are located between nucleotide -35 and +6 from the peak centers.

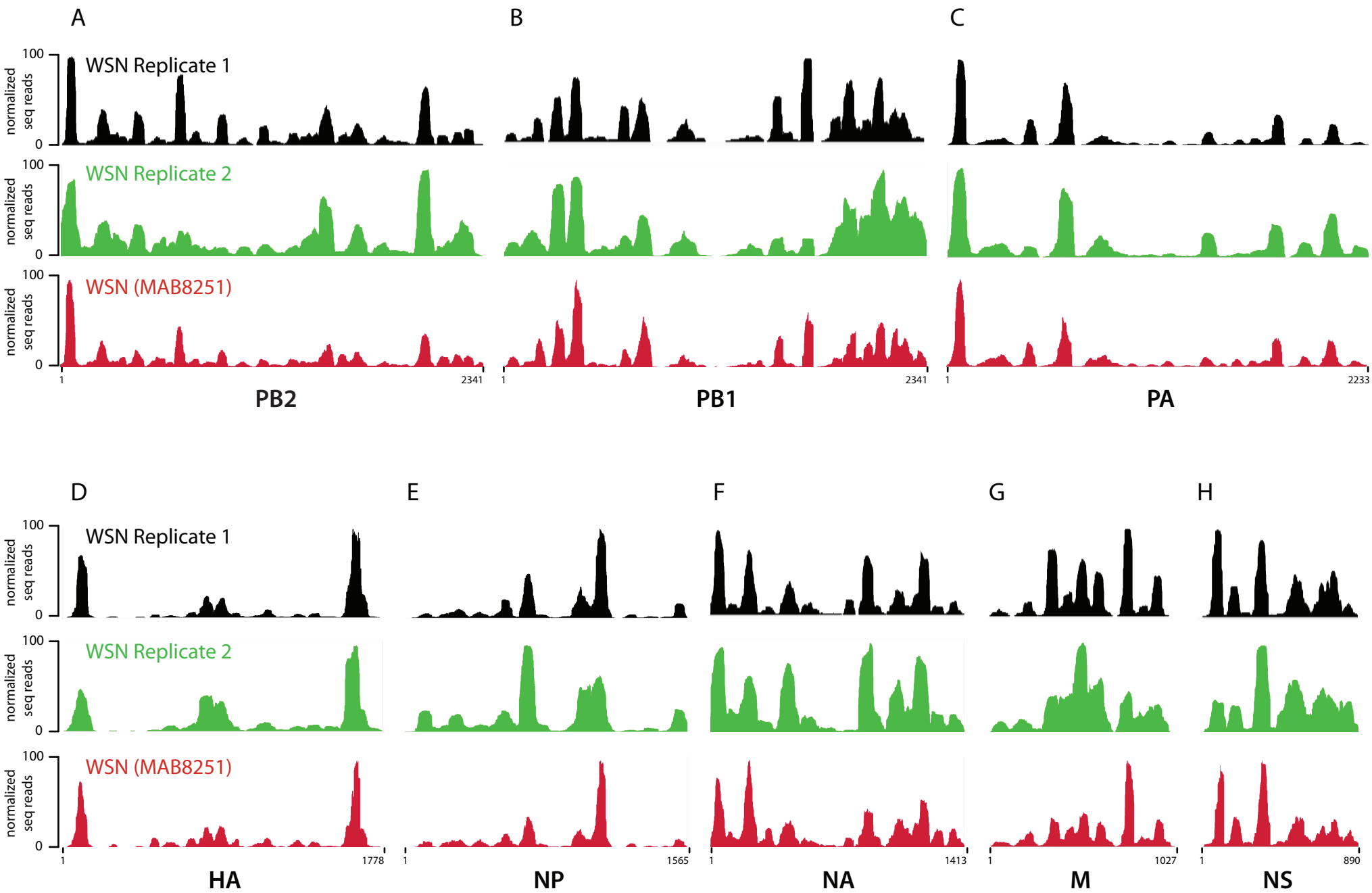
Supplementary Figure S4. Evolutionary sequence conservation is not the determinant of NP association with vRNA. (**A-B**) Sequence variability of 385 and 353 H1N1 sequences are shown for the HA (**A**) and NA segment (**B**), respectively. A sliding window algorithm of 50 bp was used to generate variability plots. Sequences corresponding to NP binding sites conserved between WSN and H1N1pdm are marked by red boxes; non-conserved peaks are displayed as solid gray boxes. No correlation between NP binding and sequence conservation is observed.

Supplementary Table S1. Pearson correlation coefficients of NP-vRNA binding profiles for all viruses and segments.

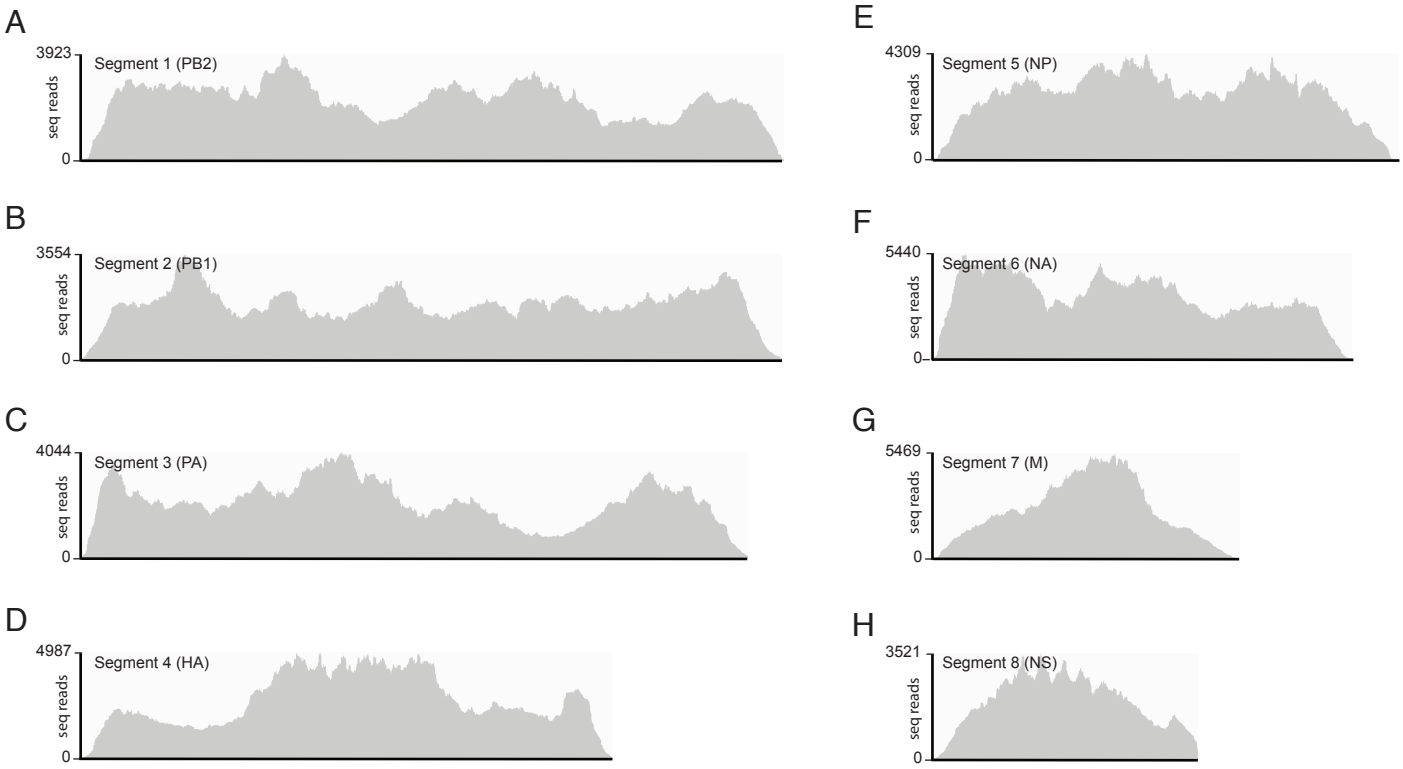
Supplementary Table S2. Summary of nucleotide contents of NP peaks and non-peaks.

Supplementary Table S3. Deep sequencing read numbers and SRA accession numbers.

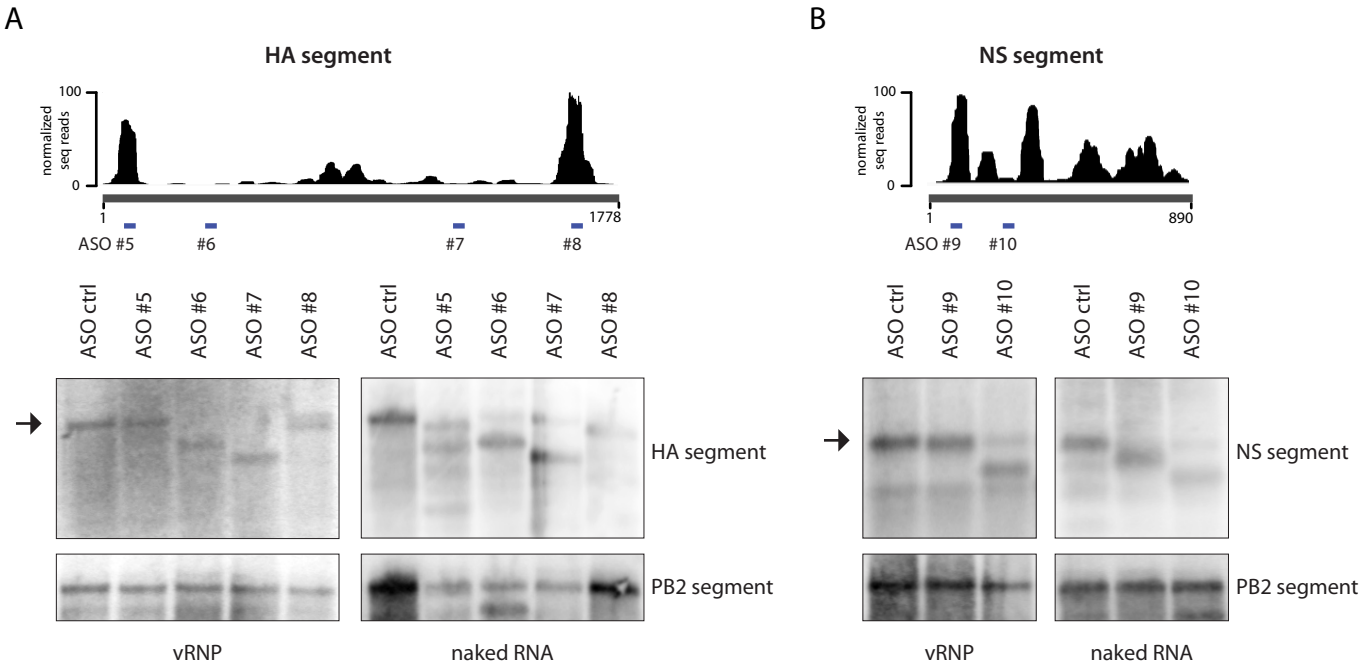
Supplementary Figure S1

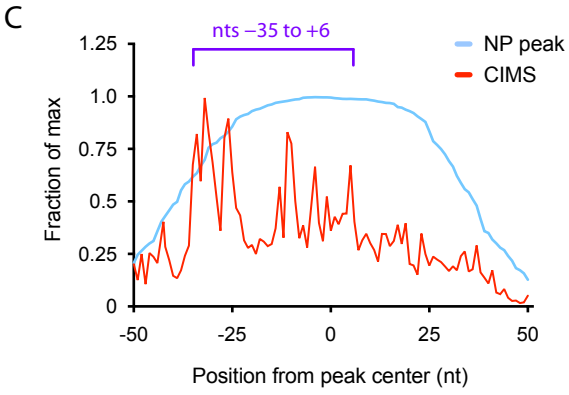


Supplementary Figure S2

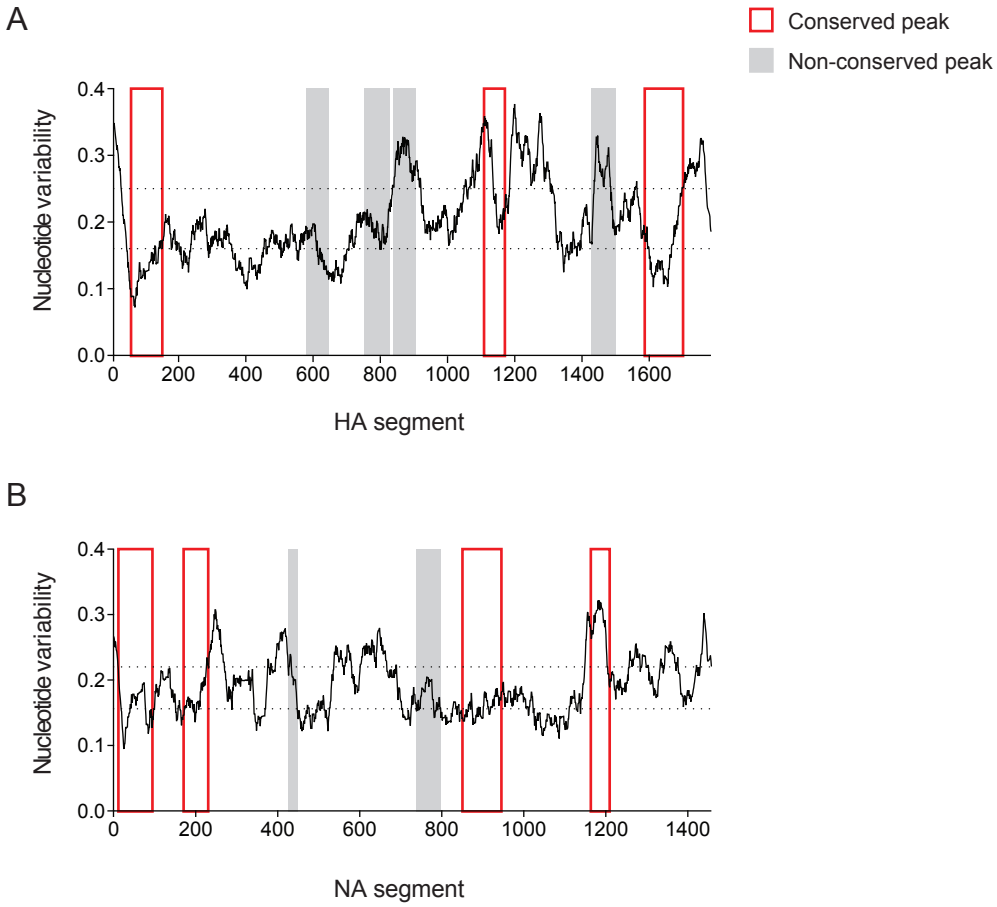


Supplementary Figure S3





Supplementary Figure S4



Supplementary Table S1

		WSN rep2		H1N1pdm		WSN 2nd Antibody	
		Pearson	p-value	Pearson	p-value	Pearson	p-value
Combined eight segments	WSN rep 1	0.798	0.0000	0.411	0.0000	0.9291	0.0000
	WSN rep 2			0.464	0.0000	0.7494	0.0000
	H1N1pdm					0.3080	0.0000
Segment 1 (PB2)	WSN rep 1	0.741	0.0000	0.399	0.0000	0.9247	0.0000
	WSN rep 2			0.397	0.0000	0.7000	0.0000
	H1N1pdm					0.3512	0.0000
Segment 2 (PB1)	WSN rep 1	0.667	0.0000	0.074	0.0004	0.8799	0.0000
	WSN rep 2			0.397	0.0000	0.7619	0.0000
	H1N1pdm					0.1335	0.0000
Segment 3 (PA)	WSN rep 1	0.884	0.0000	0.663	0.0000	0.9722	0.0000
	WSN rep 2			0.590	0.0000	0.8699	0.0000
	H1N1pdm					0.6883	0.0000
Segment 4 (HA)	WSN rep 1	0.896	0.0000	0.685	0.0000	0.9671	0.0000
	WSN rep 2			0.541	0.0000	0.8645	0.0000
	H1N1pdm					0.6993	0.0000
Segment 5 (NP)	WSN rep 1	0.747	0.0000	0.390	0.0000	0.9705	0.0000
	WSN rep 2			0.635	0.0000	0.6397	0.0000
	H1N1pdm					0.3414	0.0000
Segment 6 (NA)	WSN rep 1	0.868	0.0000	0.538	0.0000	0.9182	0.0000
	WSN rep 2			0.485	0.0000	0.7731	0.0000
	H1N1pdm					0.5187	0.0000
Segment 7 (M)	WSN rep 1	0.695	0.0000	0.344	0.0000	0.9055	0.0000
	WSN rep 2			0.693	0.0000	0.5380	0.0000
	H1N1pdm					0.1708	0.0000
Segment 8 (NS)	WSN rep 1	0.709	0.0000	0.097	0.0016	0.9366	0.0000
	WSN rep 2			0.271	0.0000	0.6957	0.0000
	H1N1pdm					–0.0205	0.5417*

* indicates statistically not significant Pearson correlation coefficient.

Supplementary Table S2

	Nucleotide	Total	NP Peaks	Non-Peaks		Mean width of peak/non-peak
WSN	A	23.7%	23.2% ± 5.5	24.2% ± 5.1		
	U	32.9%	**28.9% ± 7.7**	34.2% ± 5.2	41 peaks	62.5 ± 15.3 nt
	G	19.2%	**23.9% ± 5.1**	17.5% ± 4.3	63 non-peaks	145.0 ± 113.6 nt
	C	24.3%	23.9% ± 4.9	24.0% ± 5.4		
H1N1 pdm	A	23.1%	23.1% ± 4.4	23.3% ± 6.0		
	U	33.5%	**29.9% ± 4.9**	37.4% ± 5.9	43 peaks	72.7 ± 24.7 nt
	G	19.3%	**22.4% ± 3.9**	16.5% ± 3.3	57 non-peaks	101.2 ± 74.1 nt
	C	24.1%	24.6% ± 5.1	22.8% ± 5.5		

Supplementary Table S3

	Strain	Mapped Reads	SRA Accession No.
HITS-CLIP	WSN replicate 1 (HB65 antibody)	291130	SRR5647932
	WSN replicate 2 (HB65 antibody)	96799	SRR5647931
	H1N1 pdm	195984	SRR5647934
	WSN replicate 1 (MAB8251)	572372	SRR5647935
	WSN replicate 2 (MAB8251)	63026	SRR5647936
RNA Input	WSN	363763	SRR5647930
	H1N1 pdm	195984	SRR5647933