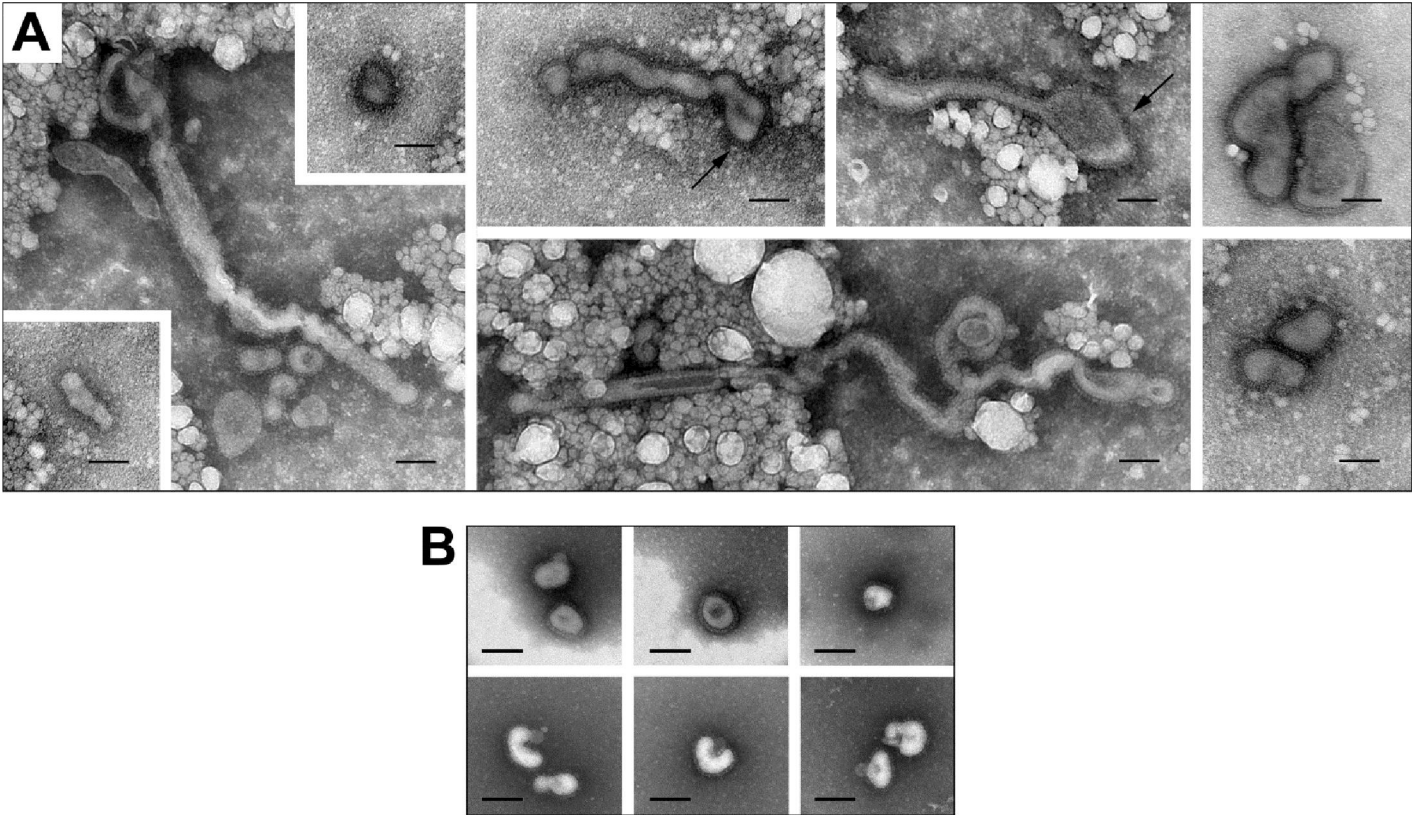


**Fig. S1. EM examination of the morphology phenotype of the rWSN virus propagated in the allantoic cavity of embryonated chicken eggs (A) or MDCK cells (B) revealed by the Amicon 100K protocol for sample preparation before negative staining. Bars: 100 nm.**

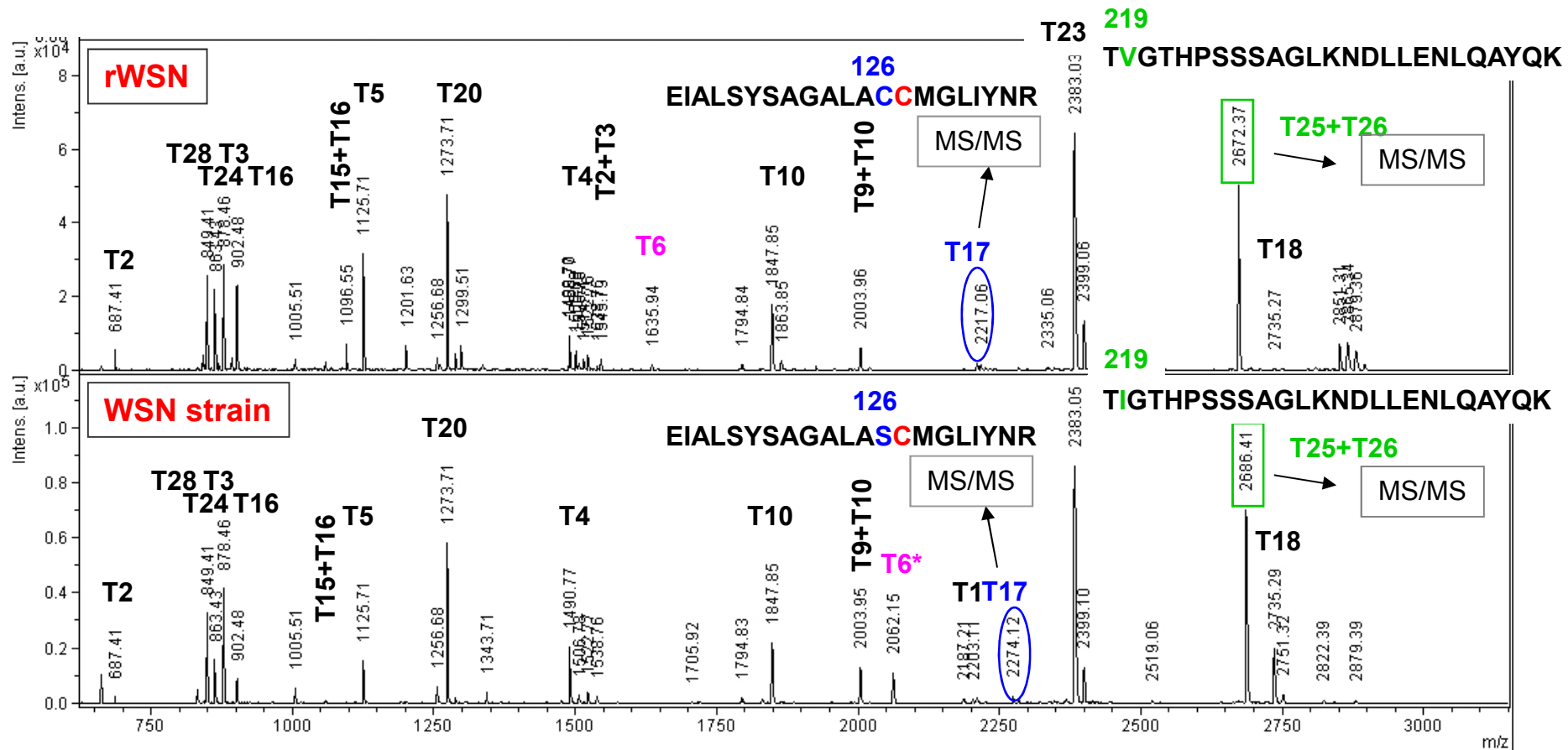


**Fig. S2. Alignment of Influenza A/WSN/33 (H1N1) virus M1 protein sequences** extracted from the Influenza Research Database ([www.fludb.org](http://www.fludb.org)) on the 9<sup>th</sup> of August, 2018.  
Two amino acid polymorphisms, **S126C** and **I219V**, are highlighted.

<a href="#">CY034133 A/WSN/1933</a>	MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRPILSPLTKGIL
<a href="#">LC333188 A/WSN/1933</a>	MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRPILSPLTKGIL
<a href="#">CY010789 A/WSN/1933 TS61</a>	MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRPILSPLTKGIL
<a href="#">M19374 A/WSN/1933</a>	MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRPILSPLTKGIL
<a href="#">L25818 A/WSN/1933</a>	MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRPILSPLTKGIL
<a href="#">X08088 A/WSN/1933</a>	MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRPILSPLTKGIL
<a href="#">MF039638 A/WSN/1933</a>	MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRPILSPLTKGIL
<a href="#">CY034133 A/WSN/1933</a>	GFVFTLTPSERGLQRRRFVQNALNGNGDPNNMDKAVKLYRKLKREITFHGAKEIALSYS
<a href="#">LC333188 A/WSN/1933</a>	GFVFTLTPSERGLQRRRFVQNALNGNGDPNNMDKAVKLYRKLKREITFHGAKEIALSYS
<a href="#">CY010789 A/WSN/1933 TS61</a>	GFVFTLTPSERGLQRRRFVQNALNGNGDPNNMDKAVKLYRKLKREITFHGAKEIALSYS
<a href="#">M19374 A/WSN/1933</a>	GFVFTLTPSERGLQRRRFVQNALNGNGDPNNMDKAVKLYRKLKREITFHGAKEIAFSYS
<a href="#">L25818 A/WSN/1933</a>	GFVFTLTPSERGLQRRRFVQNALNGNGDPNNMDKAVKLYRKLKREITFHGAKEIALSYS
<a href="#">X08088 A/WSN/1933</a>	GFVFTLTPSERGLQRRRFVQNALNGNGDPNNMDKAVKLYRKLKREITFHGAKEIALSYS
<a href="#">MF039638 A/WSN/1933</a>	GFVFTLTPSERGLQRRRFVQNALNGNGDPNNMDKAVKLYRKLKREITFHGAKEIALSYS
<a href="#">CY034133 A/WSN/1933</a>	AGALASCMGLIYNRMGAVTTEVAFGLVCATCEQIADSQHRSHRQMVTTTNPLIRHENRMV
<a href="#">LC333188 A/WSN/1933</a>	AGALASCMGLIYNRMGAVTTEVAFGLVCATCEQIADSQHRSHRQMVTTTNPLIRHENRMV
<a href="#">CY010789 A/WSN/1933 TS61</a>	AGALASCMGLIYNRMGAVTTEVAFGLVCATCEQIADSQHRSHRQMVTTTNPLIRHENRMV
<a href="#">M19374 A/WSN/1933</a>	AGALASCMGLIYNRMGAVTTEVAFGLVCATCEQIADSQHRSHRQMVTTTNPLIRHENRMV
<a href="#">L25818 A/WSN/1933</a>	AGALASCMGLIYNRMGAVTTEVAFGLVCATCEQIADSQHRSHRQMVTTTNPLIRHENRMV
<a href="#">X08088 A/WSN/1933</a>	AGALASCMGLIYNRMGAVTTEVAFGLVCATCEQIADSQHRSHRQMVTTTNPLIRHENRMV
<a href="#">MF039638 A/WSN/1933</a>	AGALASCMGLIYNRMGAVTTEVAFGLVCATCEQIADSQHRSHRQMVTTTNPLIRHENRMV
<a href="#">CY034133 A/WSN/1933</a>	LASTTAKAMEQMAGSSEQAAEAMDIASQARQMVQAMRTVGTHPSSSAGLKDDLLENLQAYQKRMGVQMQRFK
<a href="#">LC333188 A/WSN/1933</a>	LASTTAKAMEQMAGSSEQAAEAMDIASQARQMVQAMRTVGTHPSSSAGLKDDLLENLQAYQKRMGVQMQRFK
<a href="#">CY010789 A/WSN/1933 TS61</a>	LASTTAKAMEQMAGSSEQAAEAMDIASQARQMVQAMRTVGTHPSSSAGLKDDLLENLQAYQKRMGVQMQRFK
<a href="#">M19374 A/WSN/1933</a>	LASTTAKAMEQMAGSSEQAAEAMDIASQARQMVQAMRTVGTHPSSSAGLKDDLLENLQAYQKRMGVQMQRFK
<a href="#">L25818 A/WSN/1933</a>	LASTTAKAMEQMAGSSEQAAEAMDIASQARQMVQAMRTVGTHPSSSAGLKDDLLENLQAYQKRMGVQMQRFK
<a href="#">X08088 A/WSN/1933</a>	LASTTAKAMEQMAGSSEQAAEAMDIASQARQMVQAMRTVGTHPSSSAGLKDDLLENLQAYQKRMGVQMQRFK
<a href="#">MF039638 A/WSN/1933</a>	LASTTAKAMEQMAGSSEQAAEAMDIASQARQMVQAMRTVGTHPSSSAGLKDDLLENLQAYQKRMGVQMQRFK

### Fig. S3. MALDI-TOF MS analysis of M1 protein tryptic peptide hydrolyzates.

Matrix M1 proteins from two virus preparations, rWSN (Ac. MN220705) and WSN strain (Ac. MN220713) were analyzed as described in the Materials and Methods section. Identified tryptic peptides of M1 (T1, T2, T3... etc.) are designated at mass spectra above the corresponding peaks with indicated  $m/z$  values. Parent ions  $m/z$  2217 (peptide T17; rWSN) and  $m/z$  2274 (T17; WSN strain) were subjected to tandem MS analysis shown in **Fig. 4**. Parent ions  $m/z$  2672 (T25+T26; rWSN) and  $m/z$  2686 (T25+T26; WSN strain) were subjected to tandem MS analysis shown in **Supplementary Fig. S4**. Note that a new T6\* peptide ( $m/z$  20162) appears at the mass spectrum got for M1 from WSN strain as a result of R72Q substitution, while the respective T6 peptide ( $m/z$  1635) was detected at mass spectrum got for M1 from rWSN. This mutation was also suggested by the NGS analysis.



**Fig. S4. Tandem MS analysis of tryptic peptides [T25+T26] (amino acids [218-242]) of M1 from rWSN virus and WSN strain.**

The MS/MS spectra of parent ions corresponding to the peptides TVGTHPSSAGLKNDLLENLQAYQK (m/z 2672; rWSN) and TIGTHPSSAGLKNDLLENLQAYQK (m/z 2686; WSN strain) showing the detected C-terminal *y*-type ions and the deduced amino acid sequences are presented.

