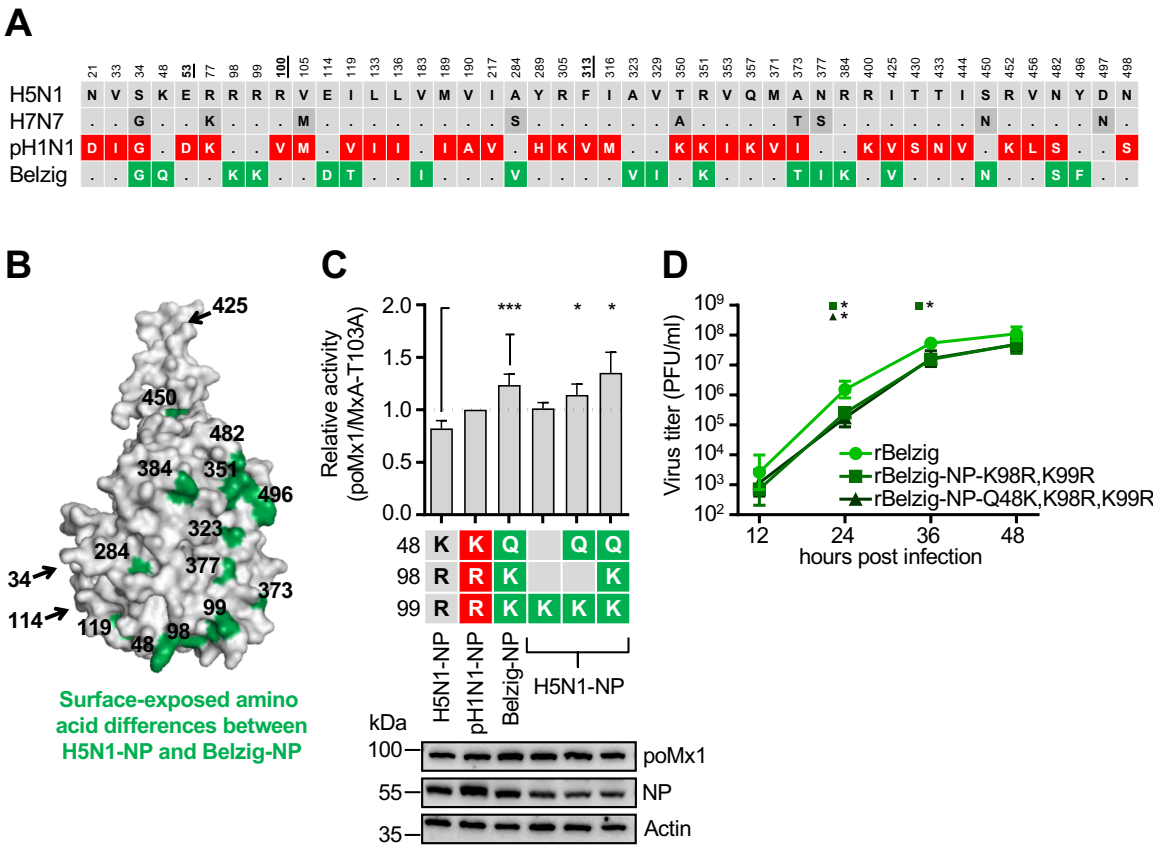
Supplemental Table 1: Conservation of amino acids in NP that confer MxA resistance. Full-length NP protein sequences of the indicated subtype and host were downloaded at 23rd of August, 2018 from [16]. In addition to 48Q, 98K and 99K, frequencies of the previously identified MxA resistance amino acids [8] are also shown. The frequency of conserved residues is indicated in %. n=number of strains analyzed.

Host	Subtype(s) /Lineage	16D	48Q	53D	98K	99K	100I/V	283P	313Y	313V	n=
Avian	All	0.1	0.1	0.1	2.4	0.0	0.3	0.1	0.1	0.1	15871
Human	H1N1 seasonal	99.9	0.0	0.0	91.5	0.1	100.0	99.7	99.9	0.0	1609
Human	H1N2/H2N2/H3N2 seasonal	100.0	0.0	0.0	11.6	0.0	99.9	100.0	100.0	0.0	12911
Swine	Classical North American swine	0.1	0.0	0.0	0.5	0.2	97.7	0.0	0.0	0.0	2842
Human	pH1N1	0.2	0.0	99.8	0.1	0.0	99.7	0.0	0.0	99.5	8998
Swine	pH1N1	0.1	0.0	22.4	0.3	0.1	94.5	0.0	0.0	98.9	1664
Human	Eurasian avian-like swine	0.0	100.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	6
Swine	Eurasian avian-like swine	0.0	97.0	0.0	96.7	97.6	0.0	0.0	0.5	0.1	737



Supplemental Figure 1: Identification of Belzig-NP amino acids with the potential to contribute to Mx resistance. (**A**) Amino acid differences between NP of H5N1, H7N7 (dark gray), pH1N1 (red) and Belzig (green). Underlined positions were previously shown to influence the MxA sensitivity phenotype. (**B**) Belzig-NP amino acid positions differing from H5N1-NP are highlighted in the structural model of A/HK/483/97(H5N1) NP (PDB code:2Q06) in green. (**C**) Polymerase reconstitution was performed as in Figure 1B but instead of 50 ng MxA-encoding plasmid 200 ng of porcine Mx1 (poMx1)-encoding plasmid was co-transfected. Expression levels of poMx1 and NP were detected via Western blot. Actin was used as a loading control. Error bars indicate the standard error of the mean of at least three independent experiments. Student's *t*-test was performed to determine the *P* value. **P*<0.05, ****P*<0.001. (**D**) NPTr cells were infected with an MOI of 0.001 with the indicated wild type or mutant Belzig viruses and incubated at 37°C. Viral titers were determined at 12, 24, 36, and 48 hours post infection via plaque assay. Error bars indicate the standard error of the mean of at least three independent experiments. Student's *t*-test was performed to determine the *P* value. **P*<0.05.

Supplemental Figure 2: Phylogenetic analysis of representative NP sequences and the presence of MxA resistance-enhancing mutations. The maximum likelihood tree of 327 aligned representative NP sequences. Nucleotide sequences were retrieved from GenBank, manually aligned and used for Bayesian tree inference with MrBayes 3.2. Substitution model: GTR+G+I. Convergence was reached after 5 million generations. The scale bar indicates substitutions per site. Avian sequences are printed in gray, human seasonal sequences (H1N1, H3N2) in blue, Eurasian porcine sequences in green, and the classical swine derived H1N1 sequences in red. Amino acid substitutions resulting in MxA resistance are indicated. Zoonotic events are highlighted with an asterisk.