Structural basis for influenza virus NS1 protein block of mRNA nuclear export

Ke Zhang1,7, Yihu Xie2,7, Raquel Muñoz-Moreno3,4, Juan Wang1, Liang Zhang5, Matthew Esparza1, Adolfo García-Sastre3,4,6, Beatriz M. A. Fontoura1* and Yi Ren2*
1–200) had no effect on this viral-mediated block and did not alter poly(A) RNA distribution when expressed in the absence of infection (Fig. 1c–e). Together, these results suggest that interaction of NS1 with overexpressed NXF1 containing the NS1-binding site released endogenous NXF1 to exert its function on nuclear export of poly(A) RNA.

To investigate the molecular determinants of NS1–NXF1–NXT1 recognition, we solved a crystal structure of the complex. Untagged full-length NS1 is prone to aggregation in solution. Mutant NS1(R38A/K41A), which suppresses NS1 aggregation but lacks RNA-binding activity13,14, was used for crystallization, as the interaction between NS1 and NXF1–NXT1 does not require RNA (Fig. 1b). We initially obtained poorly diffracting crystals using NS1(R38A/K41A) and a truncated NXF1(117–619)–NXT1 heterodimer that encompasses all annotated domains (NXF1 residues 117–619 and NXT1 residues 1–140). In light of the observation that the flexible linker connecting the RNA-binding domain (RBD) and the effector domain (ED) of NS1 causes structural polymorphism13,14, this linker in NS1(R38A/K41A) was shortened (residues 80–85 replaced by a proline residue) to obtain diffraction-quality crystals. We refer to the resulting NS1 mutant in the text as *NS1 (Supplementary Fig. 1). Importantly, the *NS1 protein remained competent to bind NXF1–NXT1 (Fig. 2f, lane 6). The *NS1–NXF1(117–619)–NXT1 complex crystallized in space group P6₃. The structure was determined by molecular replacement and refined at 3.8 Å resolution with R cryst and R free values of 23.6% and 28.3%, respectively (where R is the measure of the quality of the structural model obtained from the crystallographic data; Supplementary Fig. 3 and Supplementary Table 1). The structure reveals a 2:2:2 complex of *NS1–NXF1(117–619)–NXT1 (Fig. 2a–c). Two *NS1 molecules dimerize through their RBD domains. The mutated R38 and K41 residues in *NS1 are located in the RBD domain, which does not contact NXF1(117–619)–NXT1 directly. The *NS1 dimer, via the EDs, caps on one end of an elongated platform generated by a domain-swapped dimer of NXF1(117–619)–NXT1. In this domain-swapped dimer, two NXT1 molecules are juxtaposed; the LRR and NTF2L of each NXF1 are located in the RBD domain, which does not contact NXF1(117–619)–NXT1 residues 1–140. In light of the observation that the flexible linker connecting the RNA-binding domain (RBD) and the effector domain (ED) of NS1 causes structural polymorphism13,14, this linker in NS1(R38A/K41A) was shortened (residues 80–85 replaced by a proline residue) to obtain diffraction-quality crystals. We refer to the resulting NS1 mutant in the text as *NS1 (Supplementary Fig. 1). Importantly, the *NS1 protein remained competent to bind NXF1–NXT1 (Fig. 2f, lane 6). The *NS1–NXF1(117–619)–NXT1 complex crystallized in space group P6₃. The structure was determined by molecular replacement and refined at 3.8 Å resolution with R cryst and R free values of 23.6% and 28.3%, respectively (where R is the measure of the quality of the structural model obtained from the crystallographic data; Supplementary Fig. 3 and Supplementary Table 1). The structure reveals a 2:2:2 complex of *NS1–NXF1(117–619)–NXT1 (Fig. 2a–c). Two *NS1 molecules dimerize through their RBD domains. The mutated R38 and K41 residues in *NS1 are located in the RBD domain, which does not contact NXF1(117–619)–NXT1 directly. The *NS1 dimer, via the EDs, caps on one end of an elongated platform generated by a domain-swapped dimer of NXF1(117–619)–NXT1. In this domain-swapped dimer, two NXT1 molecules are juxtaposed; the LRR and NTF2L of each NXF1 are connected by a linker that traverses along the surface of both NXT1 molecules. The RNA recognition motif (RRM) and ubiquitin-associated (UBA) domains of NXF1 were not well ordered, and were therefore not built in the present structure. Of note, the elongated NXF1(117–619)–NXT1 platform is symmetrical. Whereas one end of the platform is capped by a NS1 dimer, the other end is unoccupied. It is therefore plausible that another NS1 dimer could bind to the unoccupied end in solution.

The NS1 dimer in the present structure is arranged asymmetrically: two NS1 EDs are oriented differently with respect to the RBD dimer (Fig. 2a–c). The NS1 dimer makes direct contacts with NXF1(117–619)–NXT1 via both EDs. One ED binds to the NXF1 NTF2L domain (interface I), whereas the other ED binds to the NXF1 LRR domain of the neighbouring NXF1 (interface II). Notably, the observed binding sites are fully consistent with our mapping of the interacting domains in NS1–NXF1–NXT1 (Fig. 1b). The *NS1–NXF1(117–619)–NXT1 structure shows that the NS1 ED employs two key phenylalanine residues, F103 and F138, to engage NXF1(117–619)–NXT1 (Fig. 2d,e). The NS1 ED adopts an α-helix β-crescent fold, with the β-strands forming a crescent-like shape around the central α-helix (Fig. 2d). Both NS1 ED and NXF1(117–619)–NXT1 do not undergo substantial conformational changes upon interaction13,14. At interface I, F103 on the α1–β2 loop of NS1 ED inserts into a hydrophobic pocket on the NXF1 NTF2L surface generated by conserved residues L383, L386, L491, P521 and L527 (Fig. 2e and Supplementary Fig. 4). At interface II, F138 on the β4–β5 loop of NS1 ED binds to the edge of NXF1 LRR, which is lined by conserved residues K213, M216, Y220, N263 and I264 (Fig. 2e and Supplementary Fig. 4). When F103 and F138 of *NS1 were mutated to alanine, individually or in combination (Supplementary Fig. 5), and analysed using in vitro binding assays with NXF1 lacking the NS1-binding site (NXF1 residues 1–110) or full-length NXF1 (residues 1–619), there was marked reduction of the interaction between *NS1 and NXF1–NXT1 (Fig. 2f). These findings corroborate the structural results regarding the critical role of these NS1 residues in the interaction with NXF1–NXT1. Importantly, F103 and F138 are highly conserved in influenza A virus NS1 proteins, and the majority of strains have at least one of these residues (Supplementary Table 2).

Comparison of the *NS1–NXF1(117–619)–NXT1 structure and the previously reported NXF1(NTF2L)–NXT1 in association with a nucleoporin FG peptide reveals that the NS1 ED and FG peptide bind to the same site on NXF1 NTF2L4 (Fig. 3a). This observation suggests that NS1 inhibits binding of FG peptide to NXF1–NXT1. We tested this hypothesis by determining whether NS1 would compete with an FG nucleoporin, Nup98, for NXF1 binding. In vitro transcribed and translated Nup98 was incubated with purified NXF1–NXT1 and increasing concentrations of purified *NS1 or *NS1(F103A/F138A) (Fig. 3b). Indeed, *NS1 protein displaced Nup98 binding to NXF1 whereas *NS1(F103A/F138A), which did not interact properly with NFX1 (Fig. 2e), was unable to alter the interaction (Fig. 3b). We then generated a recombinant influenza virus carrying the same F103A/F138A mutations on NS1 and infected cells with this mutant virus. Cell extracts were analysed by immunoprecipitation with IgG or NXF1 antibodies, followed by western blotting to detect NS1, NXF1 and Nup98 (Fig. 3c). Again, NS1(F103A/F138A) exhibited reduced interaction with NXF1 in infected cells, compared with wild-type NS1. Similarly, the interaction of the FG nucleoporin Nup153 with NXF1 was diminished in the presence of NS1, and this interaction required the C-terminal FG-repeat domain of Nup153 (Supplementary Fig. 6). These findings demonstrate a key role for NS1 F103 and F138 residues in the interaction with NXF1 in vitro and in infected cells, which prevents NXF1 docking to a subset of FG nucleoporins involved in mRNA nuclear export17–21.

To determine the effect of the virus harbouring NS1(F103A/F138A) on the intracellular distribution of poly(A) RNA, RNA FISH was performed in infected HBECs to monitor poly(A) RNA and viral M mRNA simultaneously. Both wild-type and mutant viruses did not markedly alter total poly(A) RNA levels (Fig. 4a and Supplementary Fig. 7a). The virus harbouring wild-type NS1 induced nuclear retention of poly(A) RNA compared with mock-infected cells, whereas virus harbouring NS1(F103A/F138A)—which is deficient in NXF1 binding—was unable to efficiently block poly(A) RNA in the nucleus, and substantial amounts of poly(A) RNA were exported (Fig. 4a,b). We also monitored the intracellular...
distribution of individual host mRNAs in A549 cells, which inhibit nuclear export of bulk poly(A) RNA to a lesser extent on infection (Supplementary Table 3) compared with HBECs (Fig. 1c,e). In A549 cells infected with the influenza virus A/WSN/33 strain (like the Texas strain, a H1N1 virus), 1,223 mRNAs were blocked in the nucleus 6h after infection, as determined by transcriptome analysis of nuclear and cytoplasmic fractions, and of total cell extracts compared with mock-infected cells (Supplementary Table 3).
Selected mRNA hits were validated by reverse transcription with quantitative PCR (RT–qPCR) in total cell extracts and nuclear and cytoplasmic fractions of A549 cells infected with recombinant virus expressing wild-type NS1 or NS1(F103A/F138A). As shown in Fig. 4c, the total levels of a subset of mRNAs were upregulated by viral infection in both wild-type and mutant viruses. However, the mRNAs blocked in the nucleus during infection by the virus expressing wild-type NS1 were exported in cells infected with virus encoding NS1(F103A/F138A). Of note, NS1 is known to be a multifunctional protein: F103 also contributes to NS1 interaction with the polyadenylation factor CPSF3022,23. As cellular mRNAs have different dynamics during processing, NS1 has differential effects on inhibition of biogenesis, processing and/or nuclear export of individual mRNAs at specific times during infection. For example, at 6 h after infection, the total mRNA levels of selective transcripts such as IFIT2 are not altered by wild-type or mutant viruses (Fig. 4c). However, IFIT2 mRNA is retained in the nucleus by the wild-type virus and is released in the presence of mutant virus (Fig. 4d). These results suggest that the total levels of IFIT2 mRNA have not yet been affected by a defect in poly(A) processing via CPSF30, which is likely to target it for degradation, but NS1 has already inhibited nuclear export of IFIT2 mRNA at this time point of infection. Among the mRNAs released by the mutant virus are several that encode antiviral factors or are regulated by interferon, including RIG-I (also known as DDX58)24, IFIT2 and IFIT325 (Fig. 4d). As a result of unleashing an antiviral response, viral protein levels

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**Fig. 2** | Structure of a 2:2:2 complex of *NS1–NXF1(117–619)–NXT1. **a**, Schematic of the *NS1–NXF1(117–619)–NXT1 complex. *NS1 binds to NXF1(117–619)–NXT1 through two interfaces, I and II. **b**, Overall structure of *NS1–NXF1(117–619)–NXT1 coloured as in **a, c**, Same structure as in **b** but rotated by 35° to highlight the *NS1–NXF1(117–619)–NXT1 binding interfaces. **d**, Schematic of the NS1 ED. **e**, Expanded views of interfaces I and II, viewed as in **f**, In vitro GST pull-down assays with NXF1–NXT1 and *NS1 or *NS1 with mutations on the NXF1-binding site shown in **e**, Purified GST–NXT1–His–NXF1 was incubated with purified *NS1, *NS1(F103A), *NS1(F138A) or *NS1(F103A/F138A). NS1 was detected by western blot and showed diminished interaction on mutation of the NXF1-binding site. The N-terminal domain of GST–NXF1(1–110) was used as a control. n = 3.
Fig. 3 | NS1 blocks binding of the FG nucleoporin Nup98 to NXF1–NXT1 in vitro and in influenza-infected cells. a, Structural comparison of the binding of NS1 and nucleoporin FG peptide (PDB ID 1IN5) to the NTF2L domain. b, Competition between NS1 and Nup98 for NXF1–NXT1 binding in vitro. c, Competition between NS1 and Nup98 for NXF1–NXT1 binding in influenza-infected cells. Total cell lysates from uninfected A549 cells or A549 cells infected with PR8-TX-NS NS1 wild type or PR8-TX-NS NS1(F103A/F138A) for 6 h were immunoprecipitated with NXF1 antibody or mouse IgG as control. Western blotting was performed to detect the depicted proteins. Relative concentrations of bound proteins were determined by densitometry. 

Methods

Reagents. Reagents used include: 50x Advantage Polymerase mix (Clontech, catalogue (cat.) no. 639202); dNTPs (Clontech, cat. no. 639125); BamHI-HF (New England Biolabs, cat. no. R3136T); NotI (New England Biolabs, cat. no. R0149S); Smal (New England Biolabs, cat. no. R0146S); T4 DNA Ligase (New England Biolabs, cat. no. M0202); QIAquick Gel Extraction Kit (Qiagen, cat. no. 28704); NEB 5-alpha Competent Escherichia coli (New England Biolabs, cat. no. C29871); Rosetta (DE3) Competent Cells (EMD Millipore, cat. no. 70954); SOC Outgrowth Medium (New England Biolabs, cat. no. B9020); QIAprep Spin Miniprep Kit (Qiagen, cat. no. 27106); Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher Scientific, cat. no. 13778150); RNasy Plus Mini Kit (Quagen, cat. no. 74134); Random Hexamers (50 µM) (Thermo Fisher Scientific, cat. no. N8001027); ProteoR Ntase Inhibitor (Roche, cat. no. 0335402001); SuperScript II Reverse Transcriptase (Thermo Fisher Scientific, 18044014); LIGHTCYCLER 480 SYBR GREEN I MASTER (Roche, 0470751601); LightCycler 480 Multiwell Plate 96, White (Roche, cat. no. 04729692011); NE-Per Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, cat. no. 78833); Complete EDTA-free protease inhibitor tablets (Sigma-Aldrich, cat. no. 1187580001); TransIT-X2 Dynamic Delivery System (Mirus Bio, cat. no. MR6000); SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, cat. no. 34096); 1-glutathione reduced (Sigma-Aldrich, cat. no. G4251-25G); ampicillin (Sigma-Aldrich, cat. no. A-9518); kanamycin monosulfate (Gold Biotechnology, cat. no. K-120-10); isopropyl-β-d-thiogalactoside (IPTG) (Gold Biotechnology, cat. no. I2481C50); imidazole (Sigma-Aldrich, cat. no. 56750); phenylmethylsulfonyl fluoride (PMSF) (RPI, cat. no. P20270); aprotinin (Santa Cruz Biotechnology, cat. no. sc-3595); leupeptin (Santa Cruz Biotechnology, cat. no. sc293558); peptatin A (Thermo Fisher Scientific, cat. no. BP267110); Glutathione Sepharose 4B (GE Healthcare, cat. no. 17-0756-01); Amlose Resin (New England Biolabs, cat. no. E8021S); Ni-NTA Agarose (Quagen, cat. no. 30210); Mono Q 5/50 GL (GE Healthcare, cat. no. 17-1151-01); Superdex 200 HR 10/30 (GE Healthcare, cat. no. 17-1088-01); EasyTag EXPRESS® Protein Labeling Mix, [35S], 7 mCi (PerkinElmer, cat. no. NE672007MC); T7 RibomAX Express Large Scale RNA Production System (Promega, cat. no. P1320); Protein...
Fig. 4 | Influenza virus mutant on the NXF1-binding site of NS1 allows nuclear export of host mRNAs and is attenuated. a, HBECs were mock-infected or ~100% infected with PR8-TX-NS NS1 wild type or PR8-TX-NS NS1(F103A/F138A) for 30 h. Samples were subjected to RNA FISH to detect viral M mRNA and poly(A) RNA. The dashed white squares indicate the views shown in the enlarged panels on the right. Scale bar, 10 μM. b, Quantification of fluorescence intensity in the nucleus and cytoplasm of samples in a followed by determination of nuclear-to-cytoplasmic ratios. Data are mean ± s.d. of 50 cells counted for each condition in three independent experiments. c, d, A549 cells were mock-infected or infected with PR8-TX-NS NS1 wild type or PR8-TX-NS NS1(F103A/F138A) at multiplicity of infection (MOI) of 2 for 6 h. RNA was purified from total cell lysates (c) or from nuclear or cytoplasmic fractions (d). mRNAs selected based on transcriptome analysis shown in Supplementary Table 3 were quantified by RT–qPCR. n = 3. e, Cells were infected as in c except that infection lasted for 8 h. Total cell lysates were analysed by western blotting to detect the depicted proteins. n = 3. f, Cells were infected as in c except that infection was performed for the indicated time points. Viral titres were measured in culture supernatants by plaque assays. n = 3. P values (b–d, f) were calculated using unpaired, two-tailed Student’s t-test. PFU, plaque-forming units. g, Model for NS1-mediated inhibition of cellular mRNA nuclear export.
GSHepharose 4 Fast Flow (GE Healthcare, cat. no. 17-0618-01); Hoechst 33258 (Molecular Probes); M mRNAs probes (Biosearch Technologies); ProLong Gold antifade reagent (Life Technologies, cat. no. P36930).

**Antibodies.** Influenza A virus NS1 antibody, a gift from J.A. Richt (National Animal Disease Center, Iowa) was used at a 1:10,000 dilution. β-actin monoclonal antibody (Sigma-Aldrich, cat. no. A53190) was used at a 1:5,000 dilution. His-tag monoclonal antibody (TAKARA and Clontech, cat. no. 63210) was used at a 1:5,000 dilution. Flag-M2 monoclonal antibody (Sigma-Aldrich, cat. no. F1804) was used at a 1:1,000 dilution. Horseradish peroxidase-conjugated secondary antibodies included donkey anti-rabbit and sheep anti-mouse (GE Healthcare, cat. nos NA934V and NA931V, respectively). Polyclonal rabbit anti-Nup98 was used at a 1:1,000 dilution. Mouse nuclear pore complex proteins antibody MAB414 (Abcam, cat. no. ab24609) was used at a 1:1,000 dilution. Mouse anti-M2 antibody (Thermo Fisher Scientific, cat. no. MA1-082) was used at a 1:1,000 dilution. Rabbit HA antibody (Genetex, cat. no. GTX127357) was used at a 1:1,000 dilution. Rabbit NA antibody (Genetex, cat. no. GTX125974) was used at a 1:1,000 dilution. Goat influenza A virus antisera (Meridian Life Science, cat. no. B65141G) was used at a 1:1,000 dilution. Mouse IgG (Thermo Fisher Scientific, cat. no. 31903) was diluted to 0.4 mg/ml.

**Cell culture.** Human lung adenocarcinoma epithelial cells (A549) were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich) and 1% penicillin-streptomycin. HBECs were cultured in keratinocyte serum-free medium supplemented with human recombinant insulin (Sigma-Aldrich) and penicillin–streptomycin (Gibco). HBECs were cultured in a 3% CO2 atmosphere at 37 °C.

Human lung adenocarcinoma epithelial cells (A549) were infected with PR8-TX-NS wild-type NS1 or PR8-TX-NS NS1(F103A/F138A) at MOI 2 for 6 h, 8 h, 12 h and 16 h. The culture supernatants were collected and titre was determined by plaque assay, as previously described.

**Plasmids.** Full-length NS1 (UniProtKB accession number Q9WPI6, residues 1–230) from A/Texas/36/1991 was cloned into NotI and BamHI sites of the pMALTEV vector (pMAL with TEV cleavage site) with MBP fusion at the N terminus. A mutant NS1 (UniProtKB accession number I7CAR2, residues 1–230) from A/Texas/36/1991 was cloned into NotI and BamHI sites of the pGEX-4T-1 vector modified to contain a TEV-cleavable N-terminal GST tag. Plasmids GST–*NS1(F103A), GST–*NS1, was cloned into BamHI and NotI sites of a pGEX-4T-1 vector modified to render using PyMOL (http://www.pymol.org/; Schrödinger).

**Crystallization and structure determination.** Crystals of *NS1–NXF1(117–619)–NXT1* were obtained at 20 °C by vapour diffusion in sitting drops using 1 µl protein mixture (NXF1(117–619)–NXT1 at 4 mg ml⁻¹, *NS1 at 8 mg ml⁻¹) and 1 µl reservoir solution consisting of 50 mM sodium citrate (pH 5.6), 0.15% polyethylene glycol 3350, 0.1 M NaCl and 0.1 M KCl. The crystals were transferred in three steps of increasing glycerol to a cryoprotectant solution containing 50 mM sodium citrate (pH 5.6), 6.5% PEG 3350, 0.1 M NaCl 0.1 M KCl and 22% glycerol. X-ray diffraction data were collected at the 21-ID-F Beamline at the Advanced Photon Source, Argonne National Laboratory. The crystals exhibited a long-unit cell axis of ~950 Å, which caused severe overlapping of diffraction spots. To resolve this issue, we adjusted the x-axis to position the crystals in an orientation so that the long axis was approximately parallel to the rotation axis. X-ray intensities were processed using the HKL2000 package. The best diffracting crystal yielded a 3.8-Å-resolution dataset in space group P6₁.

The asymmetric unit contains two copies of a 2:2:2 complex of *NS1–NXF1(117–619)–NXT1* at 1.25 e Å⁻³. The electron density map was obtained by molecular replacement using the coordinates of NFX1–NXT1 (PDB ID 4WKF for the NXF1-NTF2L domain and NX1T; 3R6W for the NXF1 LRR domain) and NS1 (PDB ID 4OPA for NS1 RBD and NS1 ED) as search models in Phaser. The structure was built with Coot and refined with phenix.refine. NCS restraints, secondary structure restraints, Ramachandran restraints and TLS (with each of the NS1 RBD, NS1 ED, NXF1 LRR, NXF1 NTF2L and NXT1 domains assigned to a group) were applied during the refinement. The *NS1–NXF1(117–619)–NXT1* complex consists of NXF1 (residues 205–423 and 430–549), NXT1 (residues 3–140) and NS1 (residues 2–78 and 80–222 for one molecule; residues 8–45, 88–136 and 142–202 for the other molecule). In the *NS1–NXF1(117–619)–NXT1* structure, NXF1 and NXT1 exhibit 80–85% sequence identity. In particular, the NS1 RBD dimer appears to be largely positioned by crystal packing. It is notable that a helix (residues 46–72) belonging to the yellow RBD (Fig. 2b) is not observed in the structure. Potential steric clashes between this helix and a symmetry-related molecule due to crystal packing is likely to have caused deviation of this helix from the expected position. Details of the data collection and refinement statistics are listed in Supplementary Table 1. Molecular graphics were rendered using PyMOL (http://www.pymol.org/; Schrödinger).

**GST pull-down assays.** GST or GST–NXT1 His–NXF1, or GST-tagged NXF1 domains were incubated with MBP or MBP–NS1; and GST–NXF1(1–110) or GST–NXF1(1–619) were incubated with *NS1(F103A), *NS1(F138A) or (F103A/F138A) in binding buffer (20 mM Tris, 150 mM NaCl, 1 mM DTT, 1 mM EDTA, pH 7.5) at room temperature for 30 min. Each protein (1 µM) was loaded into the binding assay. Beads were pelleted by centrifugation at 5,000 r.p.m. for 5 min and washed five times with 1 ml of binding buffer. Proteins remaining on the resin were extracted with sample buffer, resolved by SDS–PAGE and then detected by western blot using NS1 antibody.

**In vitro binding assays with Nup98 and Nup153.** Nup98 protein (UniProtKB accession number P52948) was generated by in vitro transcription and translation using a coupled reticulocyte lysate transcription–translation system in the presence of [35S]methionine, and used to test for the manufacture of high-affinity binding proteins. In vitro binding reactions between Nup98 and NXF1–NXT1 were carried out using 25 µl of in vitro transcribed and translated proteins and 2 µM GST–NXT1 His–NXF1 recombinant protein. Increasing amounts (2 µM, 4 µM and 8 µM) of *NS1 or *NS1(F103A/F138A) were added to the binding assay. Bound and unbound fractions were subjected to SDS–PAGE followed by autoradiography.
Circular dichroism. Circular dichroism (CD) spectra were collected using 12 µM of recombinant NS1 proteins (*NS1, *NS1(F103A), *NS1(F138A)) in a CD buffer (20 mM sodium phosphate pH 8.0, 50 mM NaF and 0.5 mM TCEP). The spectra were collected in the far-UV range (250–190 nm) on a Jasco-815 CD spectrometer in 1 mm path-length cuvette at a scanning rate of 50 nm/min at 0.1 nm intervals. The final CD spectrum for each sample was obtained by averaging six repeat scans. The resulting spectra are plotted as molar ellipticity against wavelength.

Statistical analysis. Statistical analyses were performed using the two-sample, two-tailed t test assuming equal variance. For statistical analysis of the M mRNA imaging study, a minimum of 50 cells were used for analysis in each condition. For all imaging studies, a one-sample Kolomogrov–Smirnov test was conducted. A normal distribution was assumed for all populations (P > 0.05).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The coordinates of the NS1–NF1–NX1 structure have been deposited in the Protein Data Bank under accession number 6ESU. All other data that support the findings of this study are available from the corresponding authors upon request.

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**Author contributions**

B.M.A.F. and Y.R. conceived the study. K.Z., Y.X., R.M.-M., J.W., L.Z., M.E. and Y.R. performed the experiments. K.Z., Y.X., R.M.-M., J.W., L.Z., M.E., A.G.-S., B.M.A.F. and Y.R. analysed the data. K.Z., Y.X., B.M.A.F. and Y.R. wrote the manuscript with input from all authors.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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|       |     | State explicitly what error bars represent (e.g. SD, SE, CI) |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

- X-ray diffraction data were collected at the Advanced Photon Source beamline 21-ID-F.
- Images for fluorescence microscopy were obtained with a Zeiss Axiovert 200 M automated microscope controlled by the AxioVision software.
- Western blot data were collected by LI-COR.

Data analysis

- X-ray diffraction data were processed and analyzed using the following programs: HKL2000, PHENIX, and COOT.
- The 2 stack images for fluorescence microscopy were deconvolved with the AutoQuant software. Pearson's correlation coefficients were analyzed by Imaris using the ImarisColoc tool.
- The signal intensity in western blots were quantified by ImageJ.

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Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
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Atomic coordinates and structure factors for the reported crystal structure have been deposited into the Protein Data Bank (PDB) under accession number 6E5U. The data for the transcriptome analysis has been deposited in GEO under accession number GSE129318. All other data that support the findings of this study are available from the corresponding authors upon request.

Field-specific reporting

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- **Sample size**: No statistical method was used to determine sample size. Sample size was designed as per prior publications in the field.
- **Data exclusions**: No data were excluded in this study.
- **Replication**: Experiments were repeated at least three times as completely independent experiments. All attempts at replication were successful.
- **Randomization**: Randomization was not relevant to this study.
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Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
|     |                       |
| X   | × Unique biological materials |
|     | Antibodies             |
| X   | Eukaryotic cell lines  |
|     | Palaeontology          |
|     | Animals and other organisms |
|     | Human research participants |

Methods

| X   | Involved in the study |
|-----|-----------------------|
|     | × ChIP-seq             |
|     | × Flow cytometry       |
|     | × MRI-based neuroimaging |

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

All unique materials are readily available from the authors or from standard commercial sources.

Antibodies

Antibodies used

- Rabbit anti-NS1 antibody
- Rabbit anti-Nup98 antibody
- Mouse anti-nuclear pore complex proteins antibody
- Mouse anti-M2 antibody
- Rabbit anti-HA antibody
- Rabbit anti-NA antibody
- Goat anti-influenza A virions antibody
- Mouse anti-NXF1 antibody
- Mouse anti-Flag antibody
- Mouse anti-β-actin antibody
- Donkey anti-rabbit antibody
- Sheep anti-mouse antibody
Validation

Rabbit anti-NS1 antibody was generated by Alicia Solórzano et al., 2005 Journal of Virology, and validated in this publication by western blot.
Rabbit anti-Nup98 antibody was generated by Aurelian Radu et al., 1995 Cell, and validated in this publication by western blot and subcellular localization.
All other antibodies were from commercial sources and were validated by suppliers.

Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) | AS49 and HBEC cells were obtained from ATCC. |
|---------------------|---------------------------------------------|
| Authentication      | RNA samples from cells were subjected to Next Generation Sequencing (NGS) and confirmed to be human cells. |
| Mycoplasma contamination | Routine mycoplasma tested showed all cell lines were free of mycoplasma contamination. |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used. |