Influenza A Virus Polymerase Recruits the RNA Helicase DDX19 to Promote the Nuclear Export of Viral mRNAs

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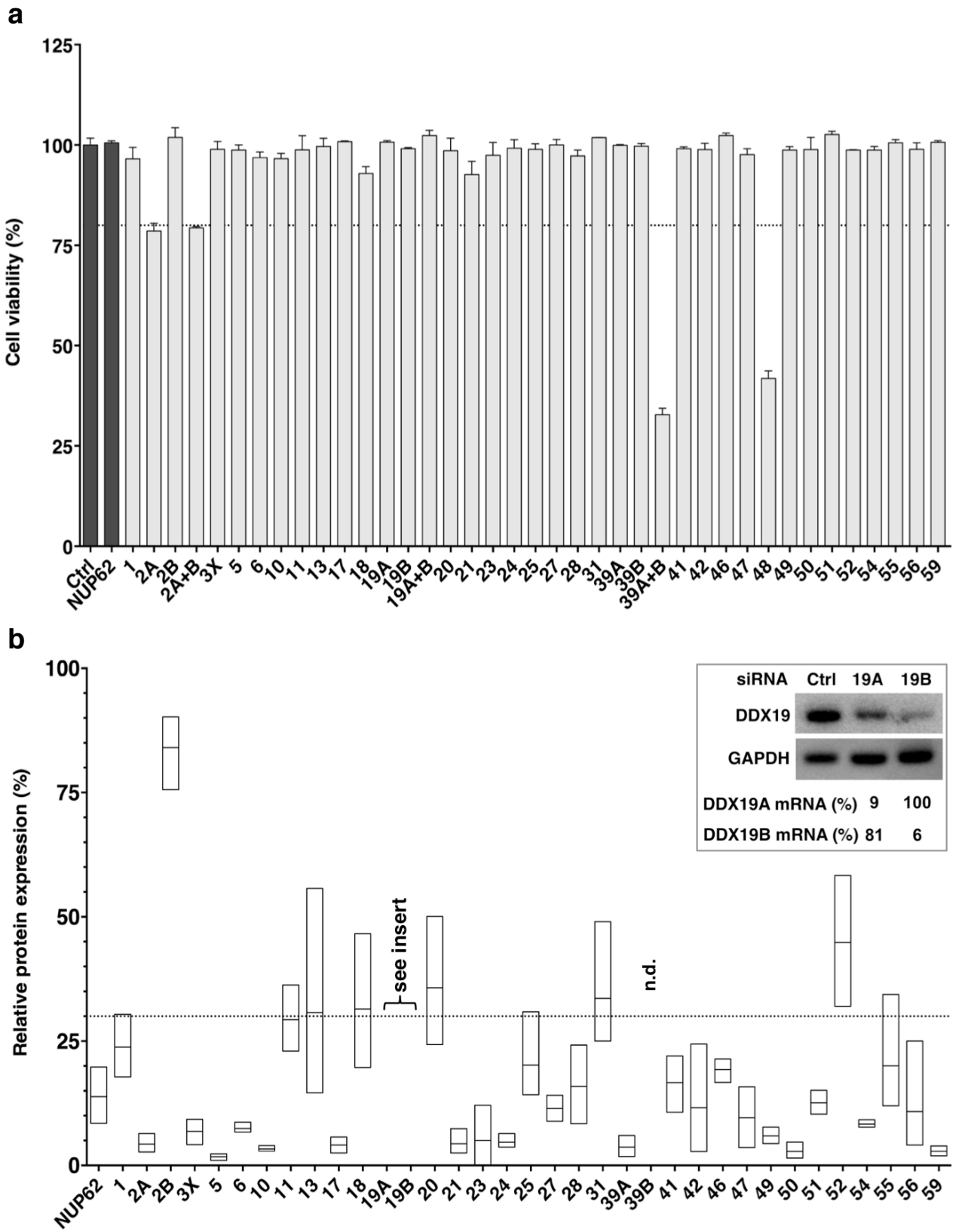
Table S1. List of human DDX proteins.

Gene Symbol	Synonyms	NCBI Gene ID	NCBI RefSeq protein	Included in the interaction screen
Bona-fide DDX proteins				
DDX2A	EIF4A1	1973	NP_001407.1	✔
DDX2B	BM-010, eIF4B, EIF4A2	1974	NP_001958.2	✔
DDX3X	DDX14, HLP2	1654	NP_001180345.1	✔
DDX3Y		8653	NP_004651.2	✗
DDX4	VASA	54514	NP_001160005.1	✗
DDX5	p68	1655	NP_004387.1	✔
DDX6	p54, RCK, HLR2	1656	NP_004388.2	✔
DDX10		1662	NP_004389.2	✔
DDX17	p72	10521	NP_006377.2	✔
DDX18	MrDb	8886	NP_006764.3	✔
DDX19A	Dbp5, Rat8	55308	NP_060802.1	✔
DDX19B		11269	NP_009173.1	✔
DDX20	DP103, Gemin3	11218	NP_009135.4	✔
DDX21	GURDB, RH-II/GU	9188	NP_004719.2	✔
DDX23	U5-100K	9416	NP_004809.2	✔
DDX24		57062	NP_065147.1	✔
DDX25	GRTH	29118	NP_037396.3	✔
DDX27	DRS1	55661	NP_060365.7	✔
DDX28	MDDX28	55794	NP_060850.2	✔
DDX31		64794	NP_073616.6	✔
DDX39A	URH49, BAT1	10212	NP_005795.2	✔
DDX39B	BAT1, UAP56	7919	NP_004631.1	✗
DDX41	ABS	51428	NP_057306.2	✔
DDX42	RHELP, SF3b125	11325	NP_031398.2	✔
DDX43	HAGE	55510	NP_061135	✗
DDX46		9879	NP_055644.2	✔
DDX47		51202	NP_057439.2	✔
DDX48	eIF4A3, eIF4A-III	9775	NP_055555.1	✔
DDX49		54555	NP_061943.2	✔
DDX50	Gu-beta, RH-II/GuB	79009	NP_076950.1	✔
DDX51		317781	NP_778236.2	✔
DDX52	ROK1	11056	NP_008941.3	✔
DDX53	CAGE	168400	NP_874358.2	✗
DDX54	DP97	79039	NP_001104792.1	✔
DDX55		57696	NP_065987.1	✔
DDX56	NOH61	54606	NP_061955.1	✔
DDX59	ZNHIT5	83479	NP_001026895.2	✔
Putative DDX proteins				
DDX1	DBP-RB	1653	NP_004930.1	✔
DDX11	CHLR1, KRG2	1663	NP_689651.1	✔
DDX12	CHLR2	440081	n.a.	✗
DDX13	SKI2W, SKIV2	6499	NP_008860.4	✔
DDX58	RIG-I	23586	NP_055129	✗
DDX60	DDX60-L	55601	NP_060101	✗


a
Cell viability (%)
125
100
75
50
25
0
Ctrl
NUP62
1
2A
2B
2A+B
3X
5
6
10
11
13
17
18
19A
19B
19A+B
20
21
23
24
25
27
28
31
39A
39B
39A+B
41
42
46
47
48
49
50
51
52
54
55
56
59

b
Relative protein expression (%)
100
75
50
25
0
siRNA Ctrl 19A 19B
DDX19
GAPDH
DDX19A mRNA (%) 9 100
DDX19B mRNA (%) 81 6
see insert
n.d.
NUP62
1
2A
2B
3X
5
6
10
11
13
17
18
19A
19B
20
21
23
24
25
27
28
31
39A
39B
41
42
46
47
49
50
51
52
54
55
56
59

Figure S1

Figure S1. Toxicity and silencing efficiency of siRNAs.

(a) A549 cells were transfected with 25 nM of siRNA and cell viability was determined at 72 hours post-transfection (hpt) using the CellTiter-Glo Luminescent Viability Assay kit (Promega). The results are expressed as the mean percentages ± SEM. When mean signals were below 80% (dashed line), siRNAs were considered as being toxic.

(b) A549 cells were transfected with 25 nM of control or DDXn-targeting siRNAs and with plasmids encoding the corresponding DDXn protein fused with the full-length *Gaussia* luciferase (pGlucFL-DDXn). Ratios of the luciferase activities obtained in cells transfected with the DDX-targeting siRNA to the one obtained in cells transfected with the control siRNA are shown. The results are represented as floating bars with a line at the mean. The dashed line corresponds to a relative protein expression of 30% in silenced cells compared to control cells. n.d.: not determined.

Insert: At 48 hpt, the levels of DDX19 protein were evaluated by immunoblot using an antibody that recognizes both A and B forms of DDX19, and the levels of DDX19A and DDX19B mRNAs were determined by RT-qPCR and expressed as percentages of the control. Cropped blots are shown. The corresponding full-length blots are shown in Figure S10.

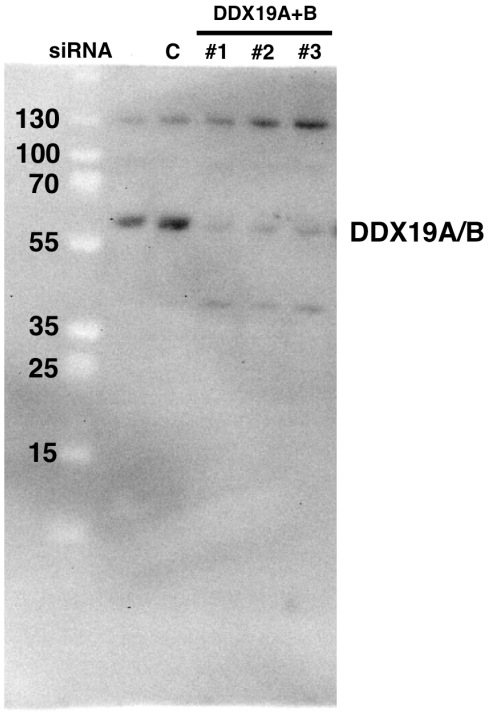


Figure S2a

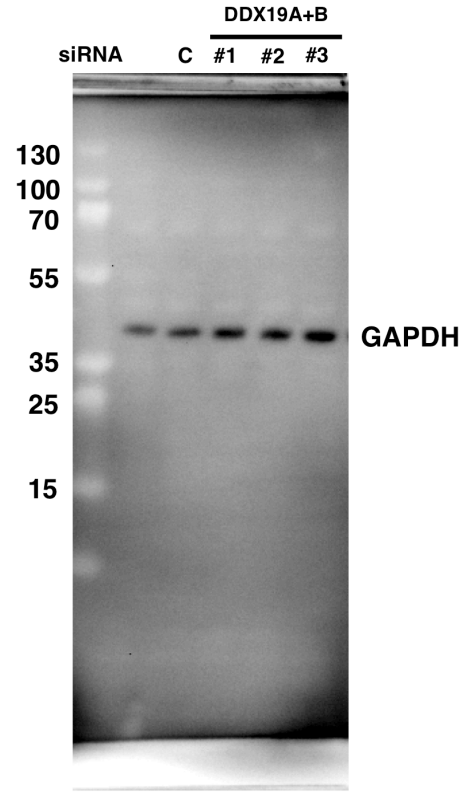


Figure S2b

Figure S2 related to Figure 1c.

A549 cells were treated with control non-target siRNAs (C) or individual siRNAs targeting DDX19A+B (#1, #2 and #3). (a) At 48 hours post-transfection, the levels of DDX19 protein were evaluated by immunoblot using an antibody that recognizes both A and B forms of DDX19. (b) The membrane was rehybridized with an anti-GAPDH antibody.

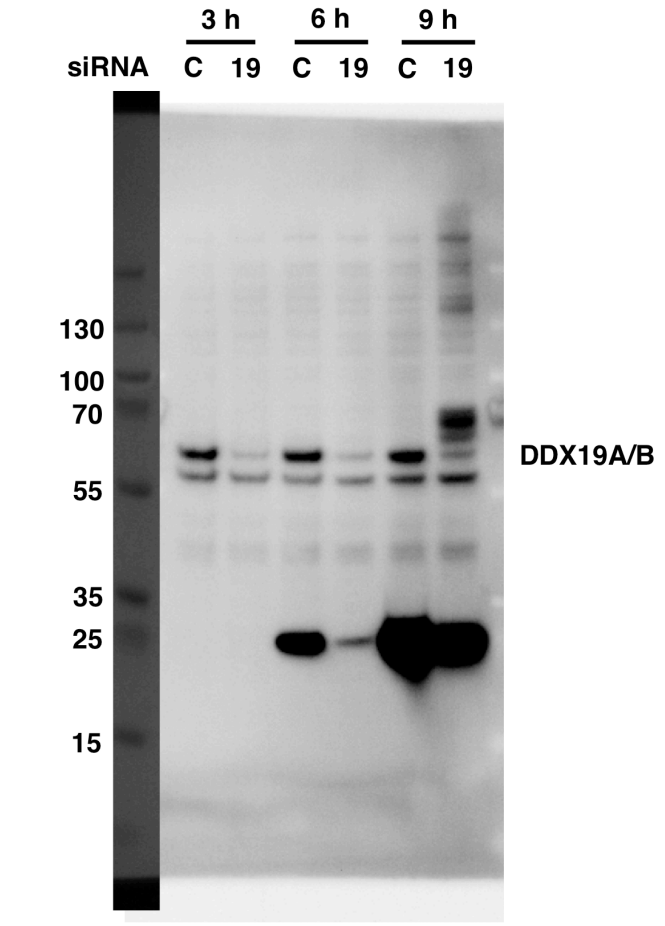


Figure S3a

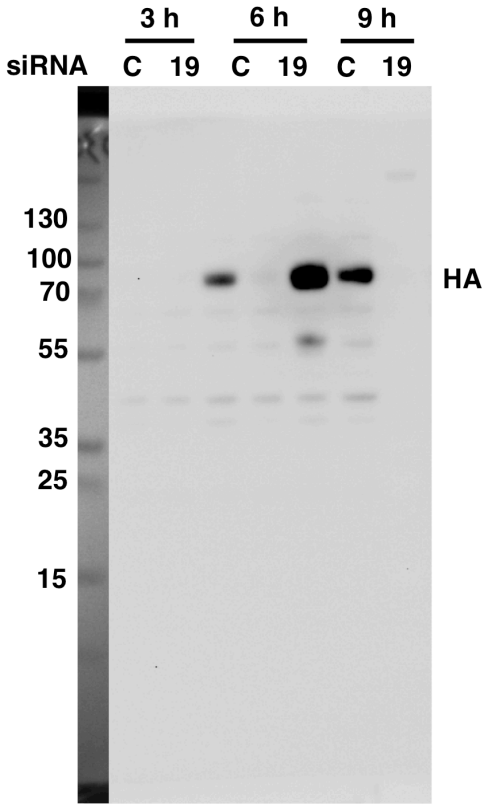


Figure S3b

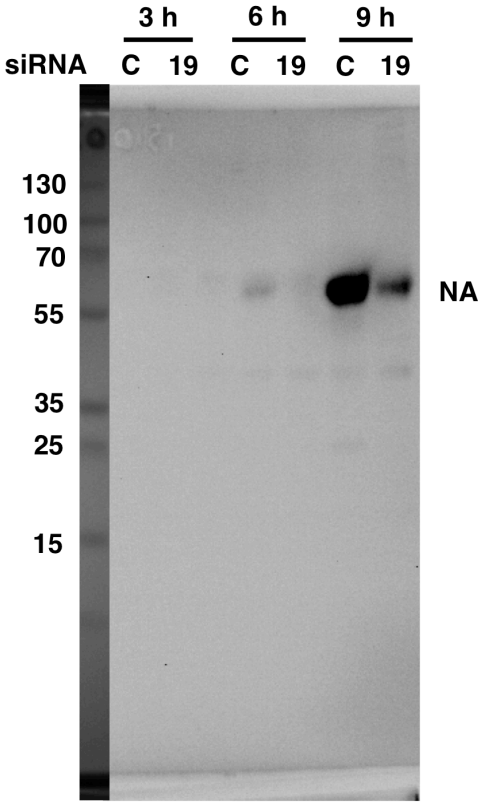


Figure S3c

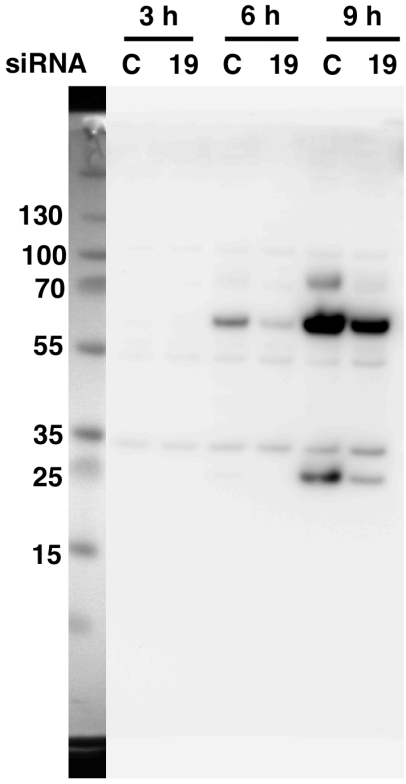


Figure S3d

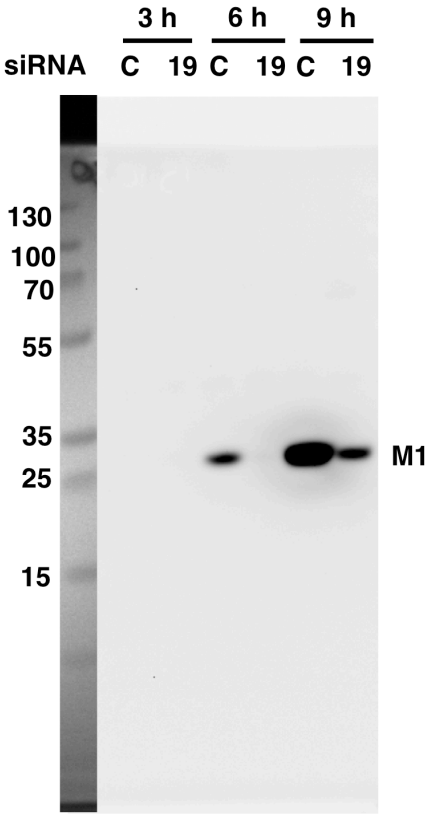


Figure S3e

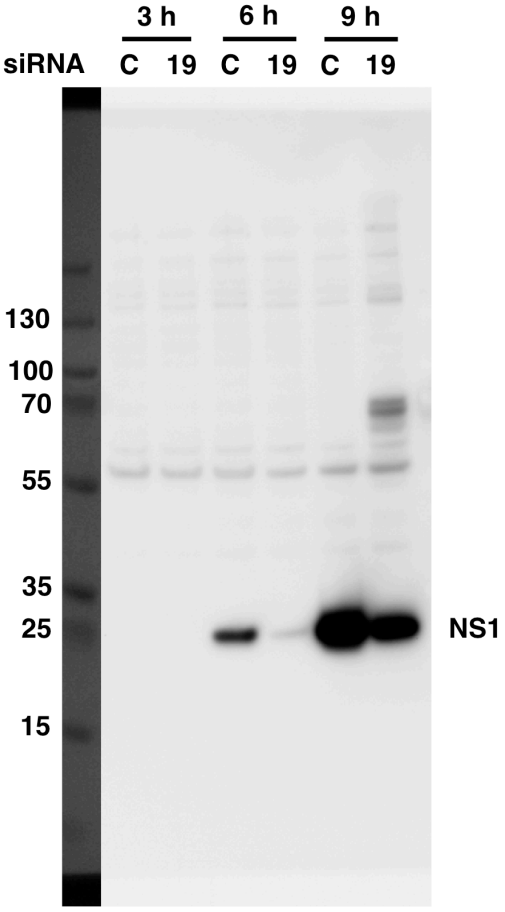


Figure S3f

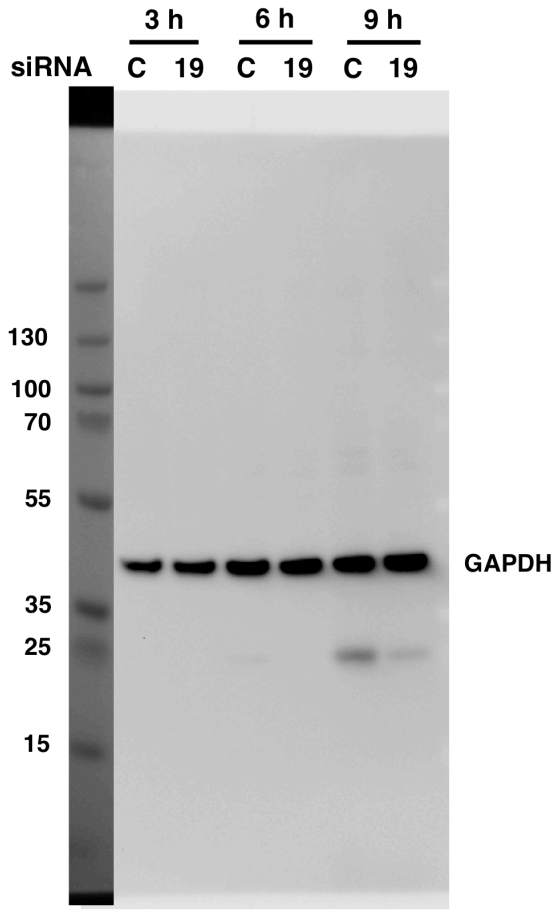


Figure S3g

Figure S3 related to Figure 2a.

A549 cells were treated with control (C) or DDX19 (19) siRNAs and infected with WSN (5 pfu/cell). Total extracts were prepared at the indicated times post-infection and analyzed by immunoblots using antibodies directed against DDX19A/B (a), HA (b), NA (c), NP (d), M1 (e), NS1 (f), or GAPDH (g). C: control siRNAs; 19: DDX19 siRNAs. Signals in (a), (f) and (g) result from serial hybridizations of the same membrane with different antibodies. Residual signals resulting from the initial hybridization with the anti-NS1 antibody (f) is visible in (a) and (g).

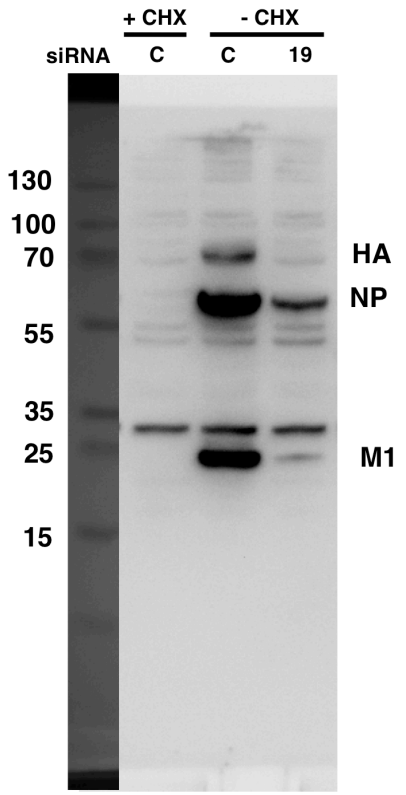


Figure S4a

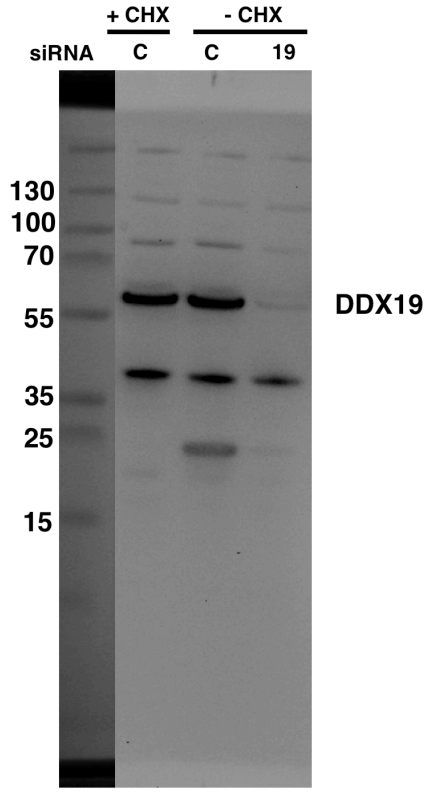


Figure S4b

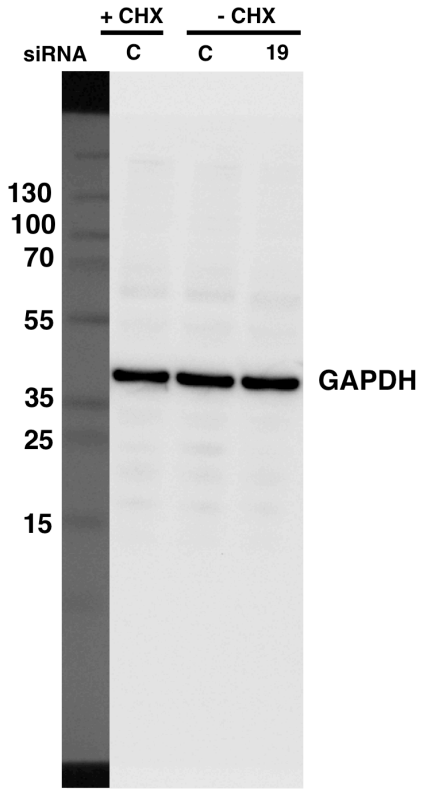


Figure S4c

Figure S4 related to Figure 3a.

A549 cells treated with control or DDX19 siRNAs were infected with WSN (5 pfu/cell) in the presence of cycloheximide (CHX; 100 µg/mL). Total extracts from control cells treated with CHX (+ CHX) or not (- CHX) were prepared at 6 hpi and analyzed by immunoblots using antibodies directed against the A/PR/8/34 virions (allowing the simultaneous detection of HA, NP and M1) (a), DDX19A/B (b) or GAPDH (c). C: control siRNAs; 19: DDX19 siRNAs. Signals in (b) and (c) result from serial hybridizations of the same membrane with different antibodies.

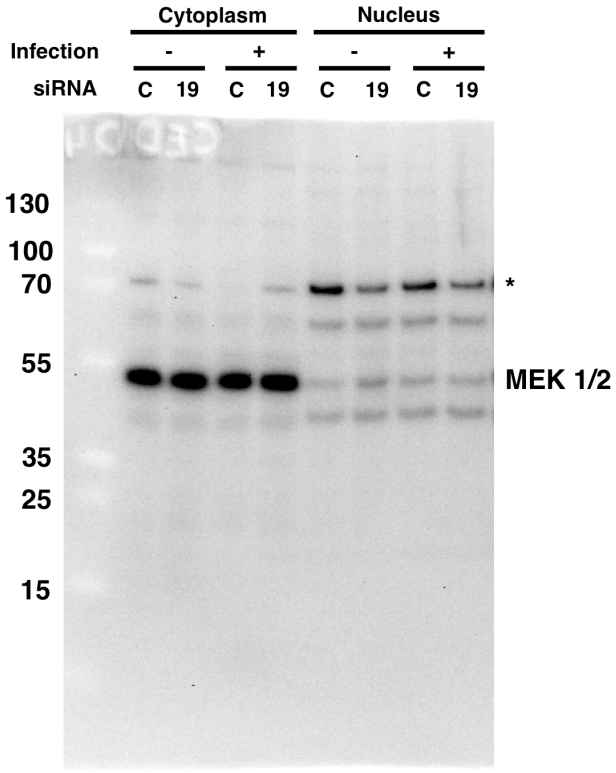


Figure S5a

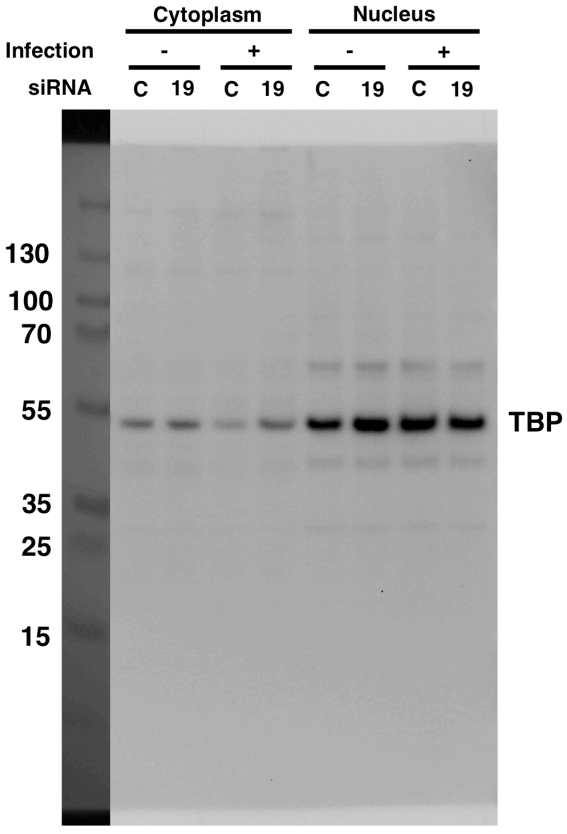


Figure S5b

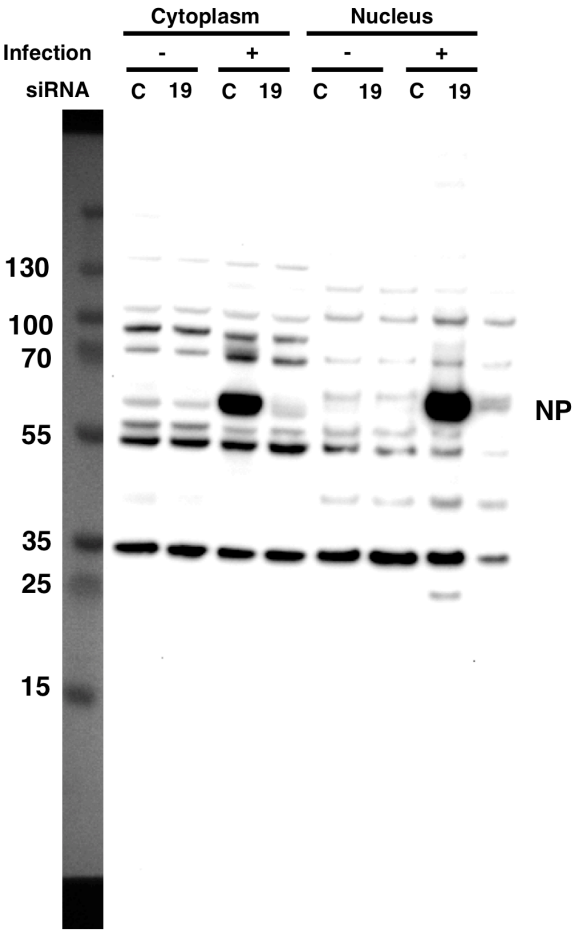

Cytoplasm
Nucleus
Infection
-
+
-
+
siRNA
C
19
C
19
C
19
C
19
130
100
70
55
35
25
15
NP

Figure S5c

Figure S5 related to Figure 3b.

Cytoplasmic and nuclear fractions from A549 cells treated with control or DDX19 siRNAs, infected or not with WSN (5 pfu/cell), were prepared. Immunoblots analysis was performed using antibodies directed against MEK1/2 (a), TBP (b) or NP (c). C: control siRNAs; 19: DDX19 siRNAs. In (a), the band indicated by a star corresponds to the lamin, as revealed upon hybridization with an anti-lamin antibody previous to the hybridization with an anti-MEK1/2 antibody.

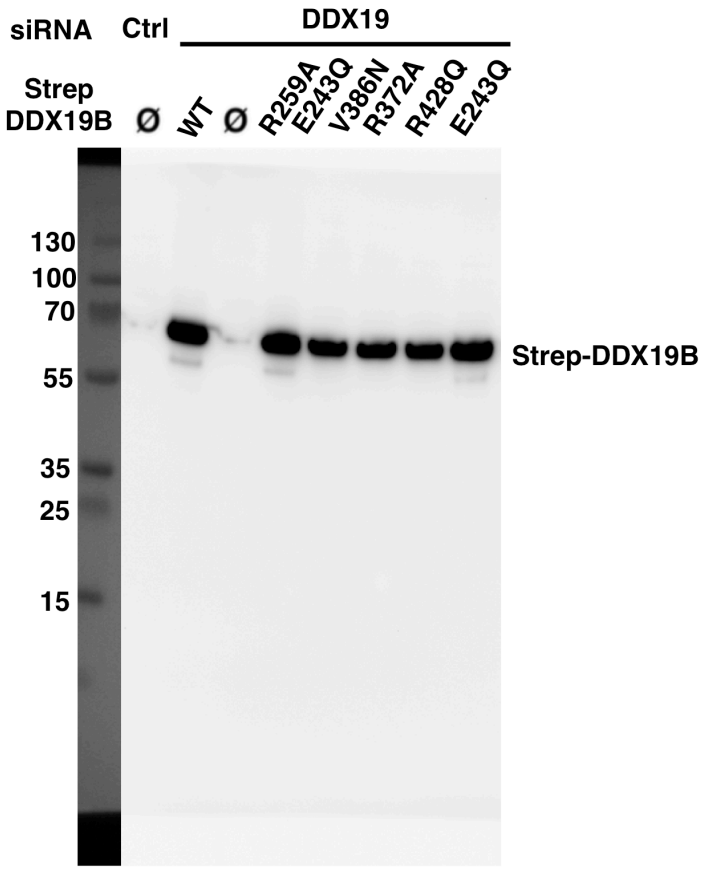


Figure S6a

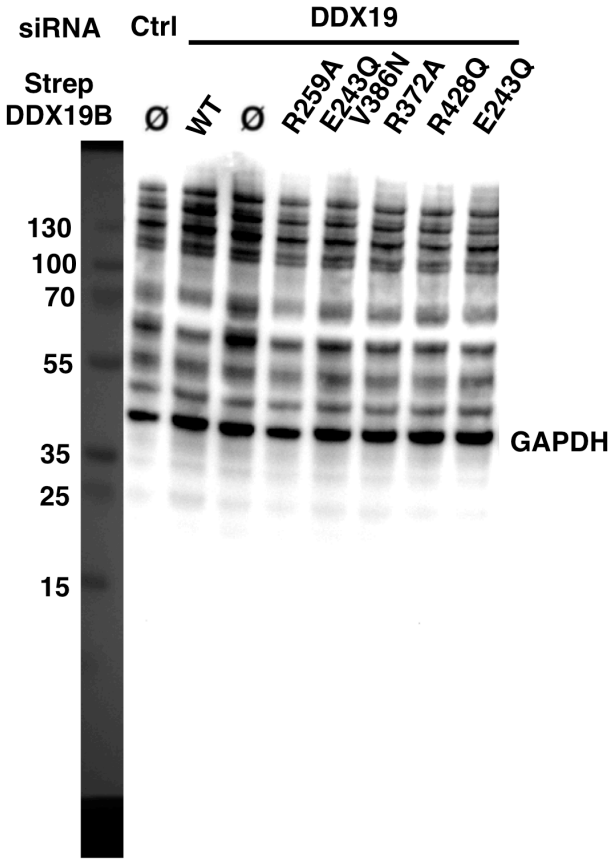


Figure S6b

Figure S6 related to Figure 4d.

HEK-293T cells were transfected with DDX19 or control siRNAs and then with siRNA-resistant DDX19B plasmids or empty vector. After 24 h, cells were infected with the WSN-PB2-Nanoluc virus (0.01 pfu/cell). (a) Lysates from the RNAi rescue experiment shown in Figure 4c were analyzed by immunoblot using Strep-Tactin. (b) The membrane was rehybridized with an anti-GAPDH antibody. The ⦸ symbol indicates cells transfected with the empty vector.

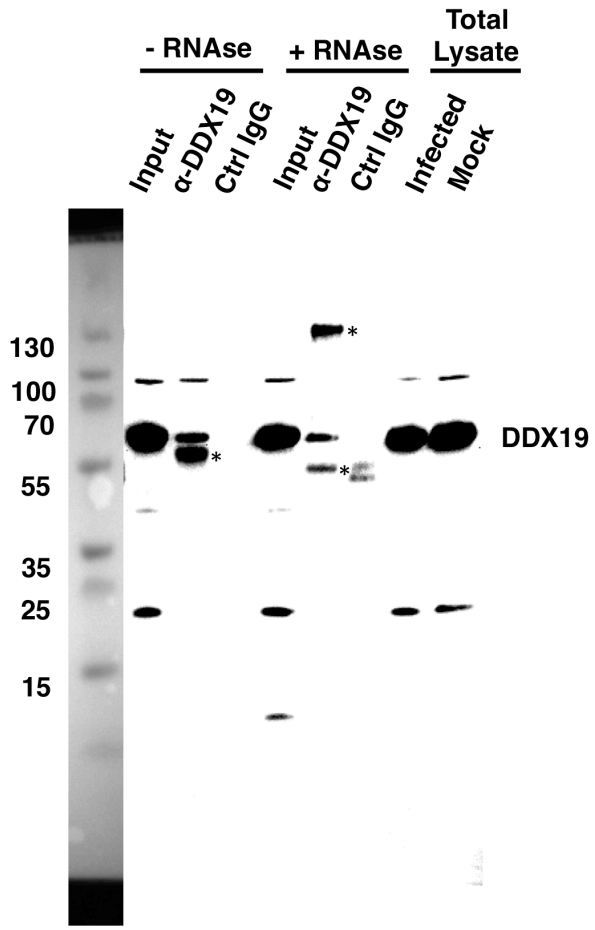


Figure S7a

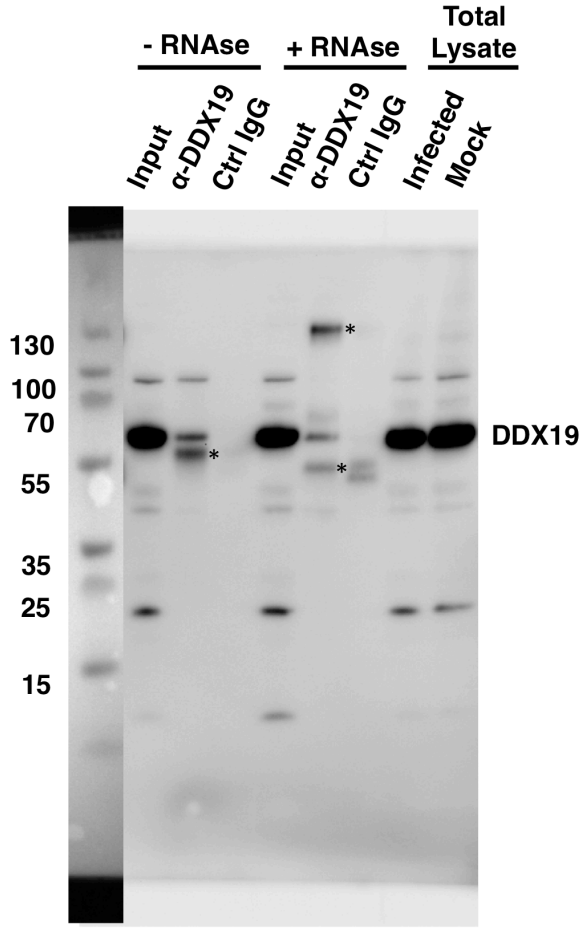


Figure S7b

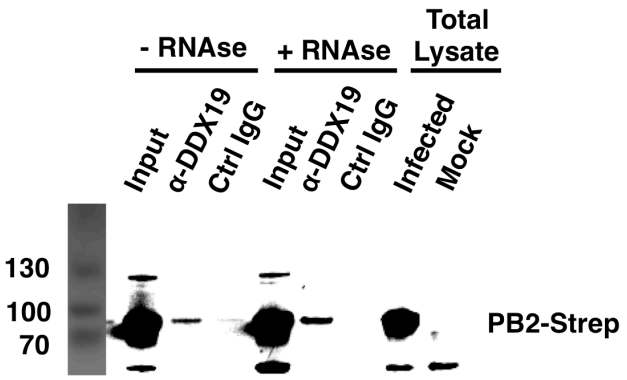


Figure S7c

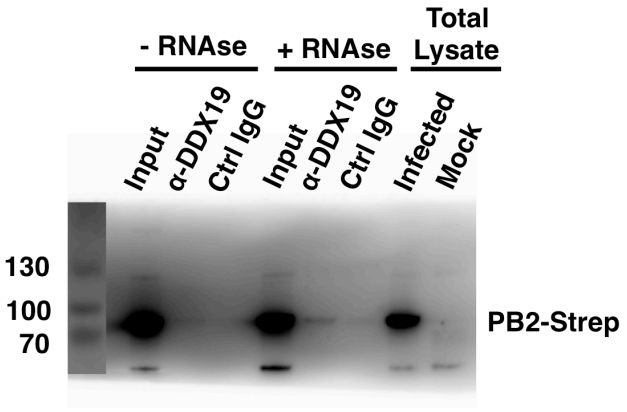


Figure S7d

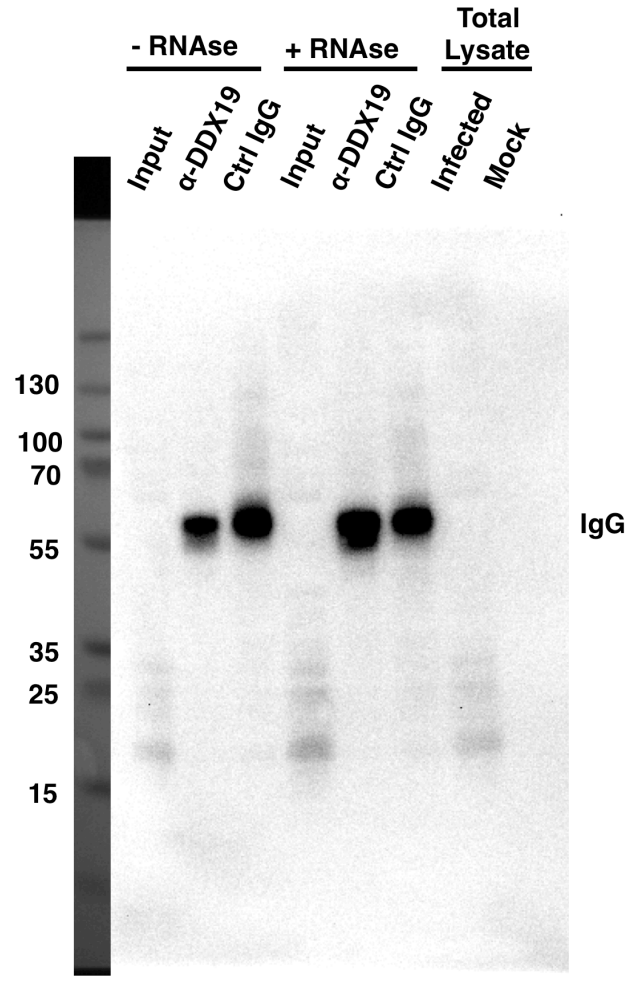


Figure S7e

Figure S7 related to Figure 5a.

A549 cells were infected with WSN (5 pfu/cell). At 3.5 hpi, DDX19 proteins were purified in the absence or presence of RNAse, using anti-DDX19 antibodies (α-DDX19) or control immunoglobulins (Ctrl IgG). Inputs and α-DDX19 / Ctrl IgG eluates were analyzed by immunoblot using anti-DDX19 antibodies (a), Strep-Tactin (c), or anti-rabbit IgG antibodies (e). (b) and (d) are identical to (a) and (b), respectively, but at a lower exposure. (c and d) Due to chemiluminescence detection reagent shortage, the membrane was cut prior to its exposure to the reagent. Results representative of two independent experiments are shown. In (a) and (b), the stars indicate bands that are not formally identified but could correspond to cellular proteins that are non-specifically immunoprecipitated and detected by anti-DDX19 antibodies, or to degradation products of DDX19 that are preferentially immunoprecipitated.

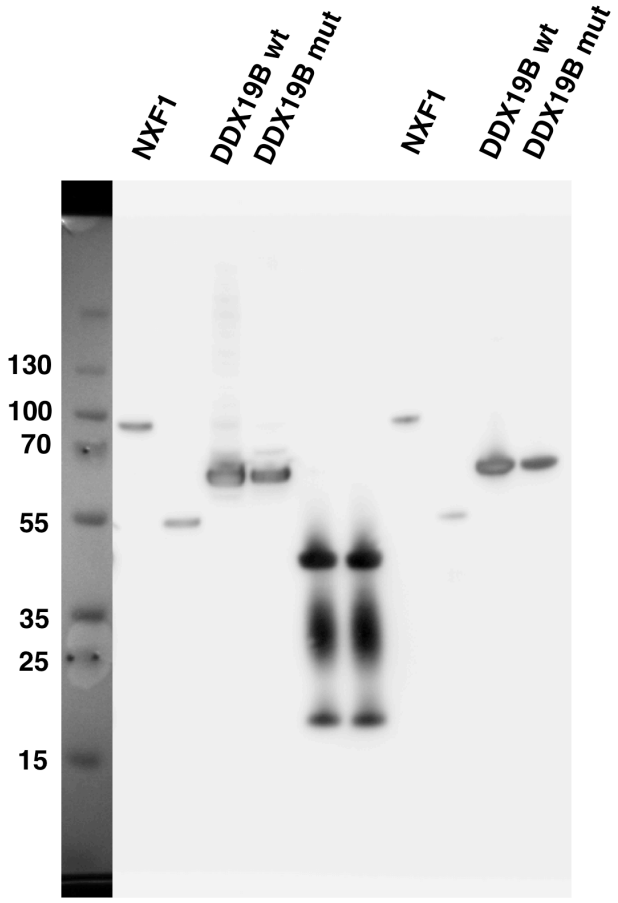


Figure S8a

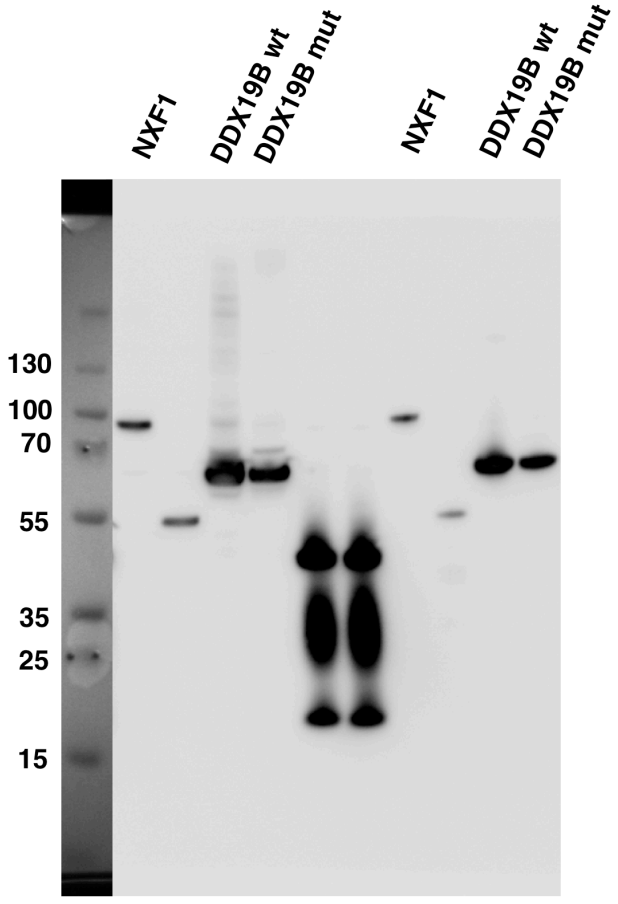


Figure S8b

Figure S8 related to Figure 5d.

(a and b) HEK-293T cells expressing Strep-tagged wild-type DDX19B, mutant DDX19B or NXF1 proteins were infected with WSN (5 pfu/cell). At 6 hpi, Strep-tagged proteins were purified. Lysates and eluates were analyzed by immunoblot using Strep-Tactin. (b) is identical to (a) but at a higher exposure.

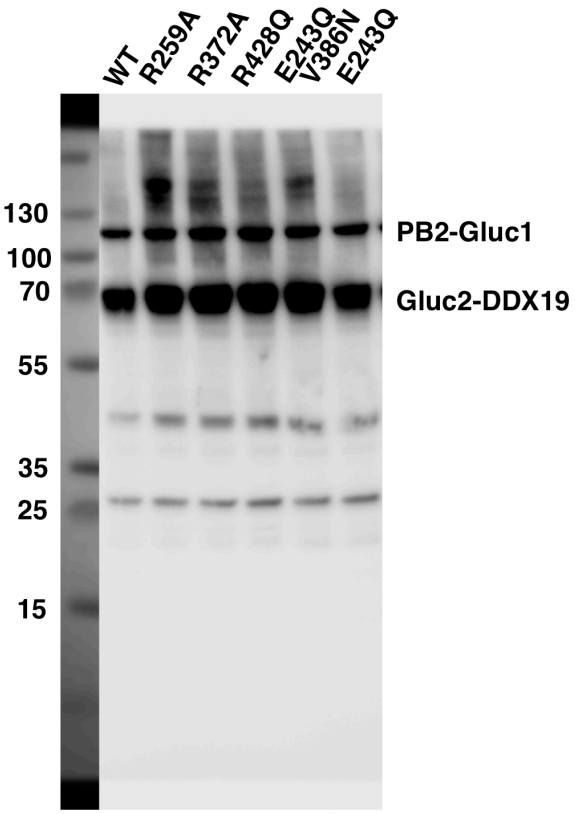


Figure S9a

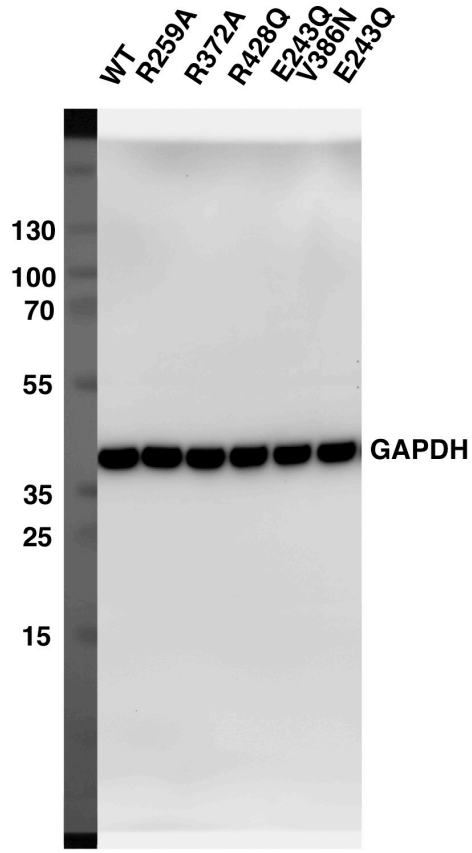


Figure S9b

Figure S9 related to Figure 5f.

HEK-293T cells expressing the Gluc2-DDX19B wild-type or mutant proteins were infected with the WSN-PB2-Gluc1 virus at a m.o.i. > 1 pfu/cell. (a) Cell lysates from the iPCA experiment shown in Figure 5e were analyzed by immunoblot using anti-Gluc. (b) The membrane was rehybridized with an anti-GAPDH antibody.

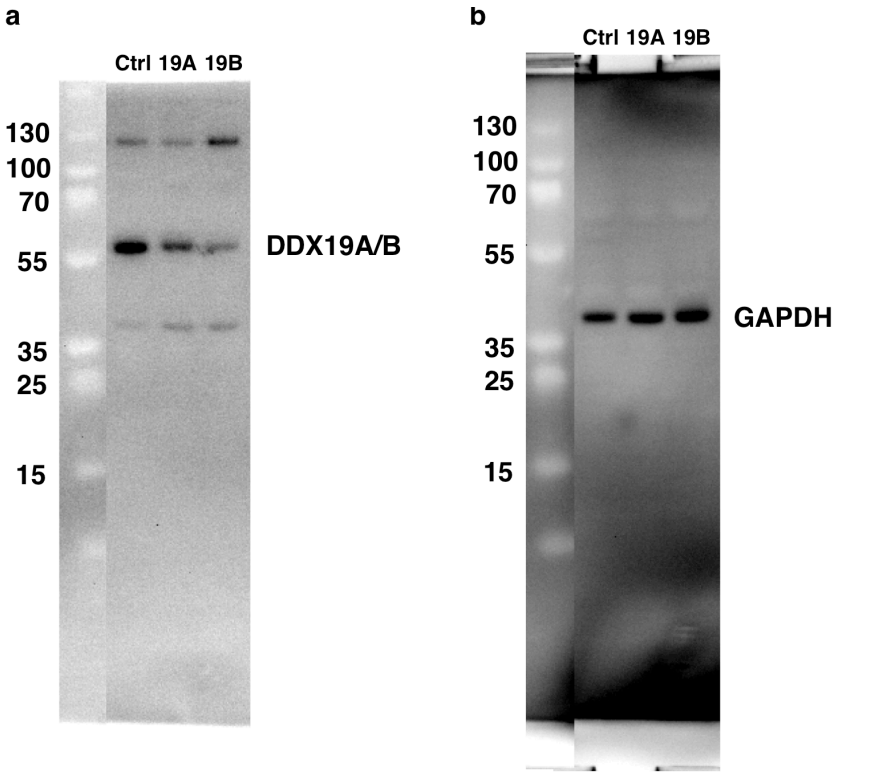


Figure S10 related to Figure S1.

A549 cells were transfected with 25 nM of control, DDX19A- or DDX19B-targeting siRNAs. (a) At 48 hpt, the levels of DDX19 protein were evaluated by immunoblot using an antibody that recognizes both A and B forms of DDX19. (b) The membrane was rehybridized with an anti-GAPDH antibody.