SUPPLEMENTAL MATERIALS

Table S1. Primers used for amplification of PB1, PB2, PA, and NP

ORF	Direction	Sequence (5'-3')
FluA-PB1	FOR	CCCCAAGCTTGATATCGCGGCCGCCACCATGGATGTCAATCCGACCTT
	REV	CGCGTCGACGGTACCTATTTTTGCCGTCTGAGCTCTT
FluA-PB2cFLAG	FOR	CGCGGATCCCGGGCGGCCGCCACCATGGAAAGAATAAA AGAACTAAGAAATCT
	REV	GCGGATCCTTATCACTTGTCGTCGTCATCCTTGTAGTCATTGATGGCCATCCGAATTC
FluA-PA	FOR	GATCCCGGGCCGCCACCATGGAAGATTTTGTGCGACA ATG
	REV	CGTAGGATCCTATTTTAATGCATGTGTCAGGAA
FluA-NP	FOR	TCGACGGTACCAGCTGAAGCTTGCTAGCGGCCGCCACCATGGCGTCCCA AGGCACCAAACG
	REV	GGAATTCATCTTAATTGTCGTACTCCTCTGCATTGT
FluB-PB1	FOR	GATCTCGAGCCGCCACCATGAATATAAATCCTTATTTTCTCTTCAT
	REV	CCCTCGAGTTATGTGTACCCAATCTCACCA
FluB-PB2cFLAG	FOR	GATCTCGAGCCGCCACCATGACATTGGCTAAAATTGAA
	REV	GCGGATCCTTACTTGTCGTCGTCATCCTTGTAGTCGCTCAAGGCCCACC
FluB-PA	FOR	GATCTCGAGCCGCCACCATGGATACTTTTAT TACAAGAAACTTCC
	REV	CCCTCGAGTTATTCATCCATTATTTCATCTACT
FluB-NP	FOR	TAGGTACCGCCGCCACCATGTCCAACATGGATATTGACG
	REV	TAATAATCGAGGTC ATCATAATCC

FOR: forward, REV: reverse

Table S2. Primers for preparation of PB2 mutants

Strain	Position	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
A/PA/99	H357W	GATAAGAGTGTGGGAGGGATAT	CTCATACCCCTCCCATACTCTTATTTTC
	H357F	GATAAGAGTATTTGAGGGGTATGAG	CTCATACCCCTCAAATACTCTTATC
	H357L	GATAAGAGTGCTTGAGGGATATG	CATATCCCTCAAGCACTCTTATC
	E361A	GGTATGCGGAGTTCACAATG	CATTGTGAACTCCGCATACC
	K376A	CTATACTCAGAGCAGCAACC	GGTTGCTGCTCTGAGTATAG
	F404W	CCATGGTGTGGTCACAAGAG	CTCTTGTGACCACACCATGG
	F404L	GCCATGGTACTGTCACAAGAG	CTCTTGTGACAGTACCATGGC
B/SH/02	Q325A	GACAAAGAGCAAGATTTGGAC	GTCCAAATCTTGCTCTTTGTC
	Q325N	GACAAAGAAACAGATTTGGACG	CGTCCAAATCTGTTTCTTTGTC
	Q325K	GACAAAGAAAGAGATTTGGACG	CGTCCAAATCTCTTTCTTTGTC
	Q325R	GACAAAGACGGAGATTTGGAC	GTCCAAATCTCCGTCTTTGTC
	E363A	GGAGAAGCAGAGTTCCATG	CATGGAACTCTGCTTCTCC
	E363D	GGAGAAGACGAGTTCCATG	CATGGAACTCGTCTTCTCC
	K378A	GGAATATTAAAAGCGAGCAAAATGAG	CTCATTTTGCTCGCTTTTAATATTCC
	W359H	GATTGGAATACATGACGGAGAAG	CTTCTCCGTCATGTATTCCAATC
	W359L	GATTGGAATACTTGACGGAGAAG	CTTCTCCGTCAAGTATTCCAATC
	W359F	GATTGGAATATTCGACGGAGAAG	CTTCTCCGTCGAATATTCCAATC
	F406Y	GTGCATGGTATACTCTCAAGAC	GTCTTGAGAGTATACCATGCAC
	F406L	TGCATGGTACTGTCTCAAGAC	GTCTTGAGACAGTACCATGCA

Figure S1. The comparison of the capped RNA cleavage activity among FluA strains and among FluB strains. (A) Proteins in purified virions (lanes 1 and 3) and vRNP (lanes 2 and 4) of FluA (lanes 1 and 2) and FluB (lanes 3 and 4) were separated through 11% SDS-PAGE containing 4 M urea and stained with CBB. (B) *In vitro* capped RNA cleavage reactions were performed using 600 ng of FluA (lanes 2 to 4) and FluB (lanes 5 and 6) vRNP with m^7GpppGm-RNA (lanes 1 to 6). Synthesized RNA products were analyzed by 15% PAGE containing 8 M urea. PA, A/Panama/2007/99 (H3N2); BJ, A/Beijing/262/95 (H3N2); PR, A/Puerto Rico/8/34 (H1N1); SH, B/Shanghai/361/2002; and Mie, B/Mie/1/93. The input capped RNAs (33 nt) and cleaved capped RNA products are indicated as closed and open triangles in the right of panel, respectively.

Figure S2. Characterization of RNA elongation reaction products. (A) The elongated products corresponding to eight full-length segments. The vRNA templates purified from FluA (lane 1) and FluB (lane 2) vRNPs were analyzed by 4% PAGE containing 8 M urea, and then detected by silver staining. The elongated products synthesized in *in vitro* RNA elongation reaction were analyzed by 4% PAGE containing 8 M urea, and then detected by autradiography (FluA: lane 3, FluB: lane 4). (B) Detection of the poly(A)$^+$ elongated products. The poly(A)$^+$ and poly(A)$^-$ elongated products (FluA: lanes 1 to 3, FluB: lanes 4 to 6) were separated using Oligotex^{TM}-dT30 <Super> (TaKaRa) according to the manufacturer's instruction. A cap1-poly(A) (as a positive control for poly(A)$^+$, 33 nt) and 53-merVwt (as a negative control for poly(A)$^-$, 5'-AGUAGAAACAAGGGUGUUUUUUCAUAUCAUUUAAACUUCACCCUGCUU UUGCU-3') (4) were also subjected to poly(A) selection and analyzed by 10% PAGE

containing 8 M urea.

Figure S3. Effect of mutations at the ribose stacking residue (Phe323) in FluA PB2 on the transcription activity. The transcription activity in the mini-replicon system was measured as FIG. 4. The firefly luciferase activity was normalized as that relative to the renilla luciferase activity. These results are averages with SD from four independent experiments.

Figure S4. The model for the interaction of m^7GTP with the cap-binding domain of FluB PB2. The models of wild type (A), Glu363Asp mutant (B), and Gln325Arg and Glu363Asp double mutant (C). In the case of wild type, the stacking interaction (purple circle) between Trp359 and methylated base and hydrogen bonds (green dotted lines) between Glu363 and guanine base and between Gln325 and ribose of guanosine forms the pocket for cap recognition. The Asp363 replaced for Glu363 could make no or weak hydrogen bonds with guanine possibly due to the longer distance (B). Arg325 replaced for Gln325 could generate hydrogen bonds with ribose more stably than Gln325. Alternatively, the interaction between Arg325 and ribose may relocate guanine base so as to make the interaction with Asp363 more stable (B).

Figure S5. The conserved mode of m^7G binding by four cap-binding proteins. The structures of four cap-binding domains with m^7G binding are drawn as relative configuration of two aromatic residues sandwiching the methylated base and the acidic residue interacting with the N1 and/or N2 positions of the guanine. (A) Influenza A virus polymerase PB2 cap-binding domain (1) (pdb entry code 2vqz), (B) Human

translation initiation factor, eIF4E (5, 6) (pdb entry code 1ipc), (C) 20 kDa human nuclear cap-binding protein, CBP20 (3) (pdb entry code 1h2t), and (D) Vaccinia virus (nucleoside-2'-*O*-)-methyltransferase, VP39 (2) (pdb entry code 4dcg).

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