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Supplementary Materials for

Human TRA2A determines influenza A virus host adaptation by regulating viral mRNA splicing

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SUPPORTING INFORMATION

Supplementary Figures

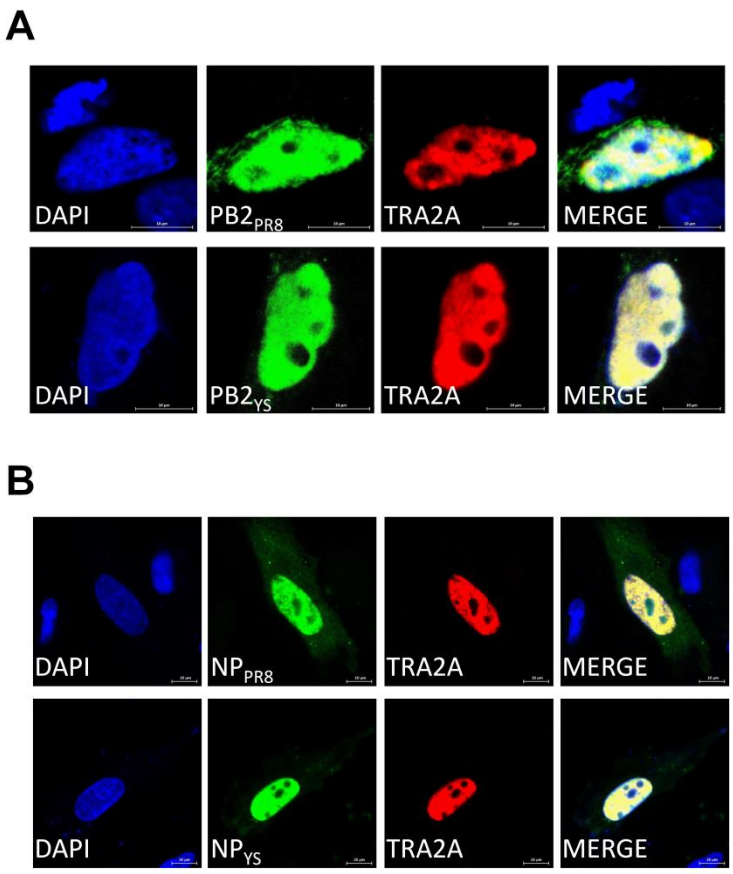

fig. S1. TRA2A colocalizes with PB2 and NP protein in nucleus. (A, B) A549 cells were cotransfected with the HA-TRA2A and Flag-$PB2_{YS}$ or Flag-$PB2_{PR8}$ (A). A549 cells were cotransfected with the HA-TRA2A and Flag-NP_{YS} or Flag-NP_{PR8} (B). After 24 h, cells were fixed and stained with the anti-HA and the anti-Flag antibody, and then visualized by confocal microscopy. The image is representative of 20 cells. Scale bar, 10 μm.

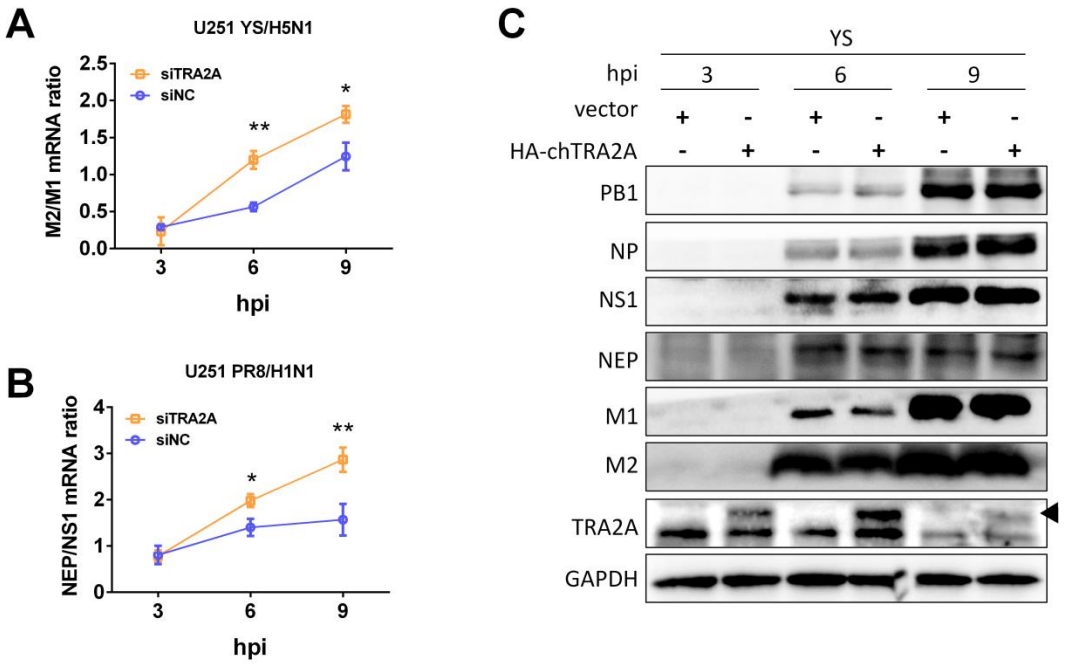

fig. S2. huTRA2A regulates viral mRNA splicing. (**A, B**) U251 cells were transfected with siNC or siTRA2A for 24 h, then infected with YS or PR8 virus at an MOI of 5. M2/M1 (A) and NEP/NS1 (B) mRNA levels were analyzed by RT-qPCR. (**C**) A549 cells were transfected with pCAGGS vector or HA-chTRA2A plasmid for 24 h, then infected with YS virus at an MOI of 5. Cell lysates were analyzed by western blot. *, $p<0.05$; **, $p<0.01$.

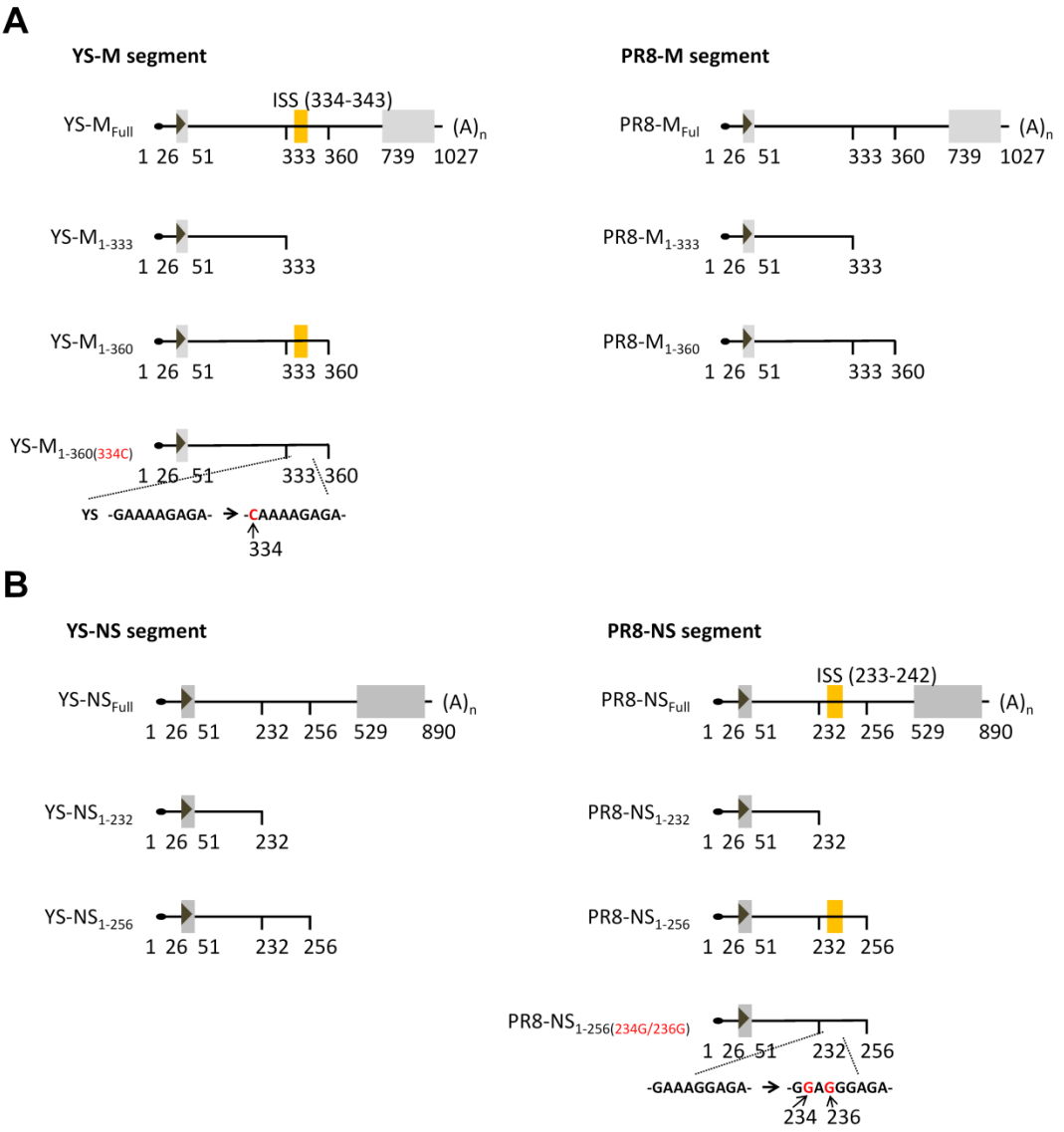

fig. S3. Truncations of YS-M/NS and PR8-M/NS segment. (A, B) Diagrams of M (A) and NS (B) segment-derived RNA substrates used for RNA pulldown assays. Names of each probe are indicated on the left. Boxes denote exons (not including the orange boxes) and the lines denote introns. Mutations of WT nucleotides are in red. The ISS motif is orange.

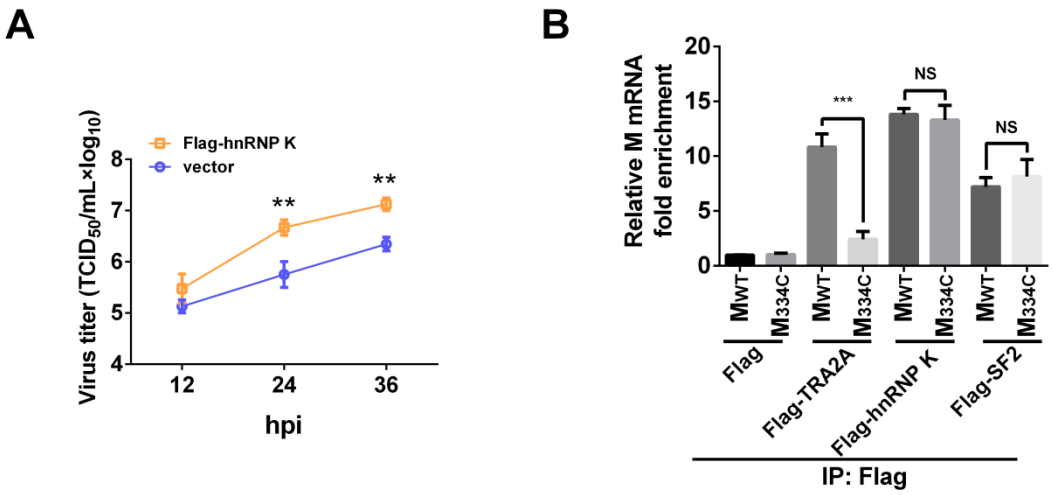

fig. S4. SR and hnRNP family proteins in RNA binding and regulation of avian virus replication. (**A**) A549 cells transfected with indicated plasmids for 24 h and then infected with either the wild type or mutant of YS virus at an MOI 0.01 for the indicated time periods. The growth curves were determined by $TCID_{50}$ analysis on MDCK cells. (**B**) HEK 293T cells were co-transfected with indicated plasmids with a pol-II driven M_{WT} or M_{334C} mutant plasmid. mRNAs were purified with the Flag antibody. The purified RNAs were then analyzed by specific RT-qPCR assay.

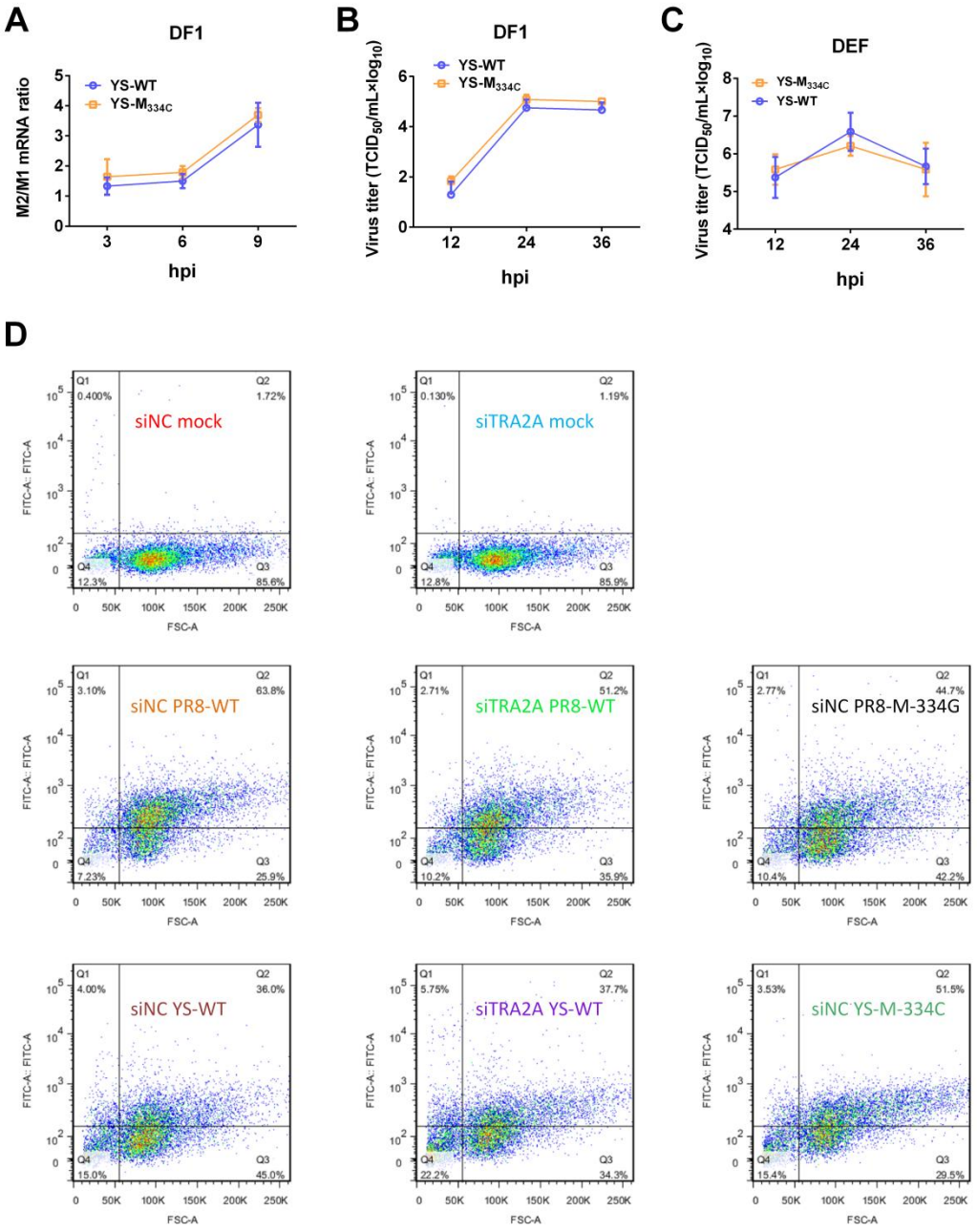

fig. S5. Avian TRA2A did not change YS virus replication and mRNA splicing. (**A**) DF1 cells were infected with the YS-WT or YS-M_{334G} virus at an MOI of 5, the total RNA was isolated and analyzed by specific RT-qPCR targeting to M1 or M2 mRNA. Ratios of M2/M1 mRNA over time are presented. (**B, C**) DF1 and DEF cells were infected with the YS-WT or YS-M_{334G} virus at an MOI of 0.01 for the indicated time periods. The growth curves were determined by $TCID_{50}$ analysis on MDCK cells. (**D**) A549 cells were transfected with siNC or siTRA2A for 24 h, then infected with either WT or mutant YS or PR8 virus for 6 hours. Cells were stained with rabbit anti-HA

antibody followed by FITC-goat anti-rabbit IgG, and analyzed by flow cytometry (Results summarized in Fig. 5K).

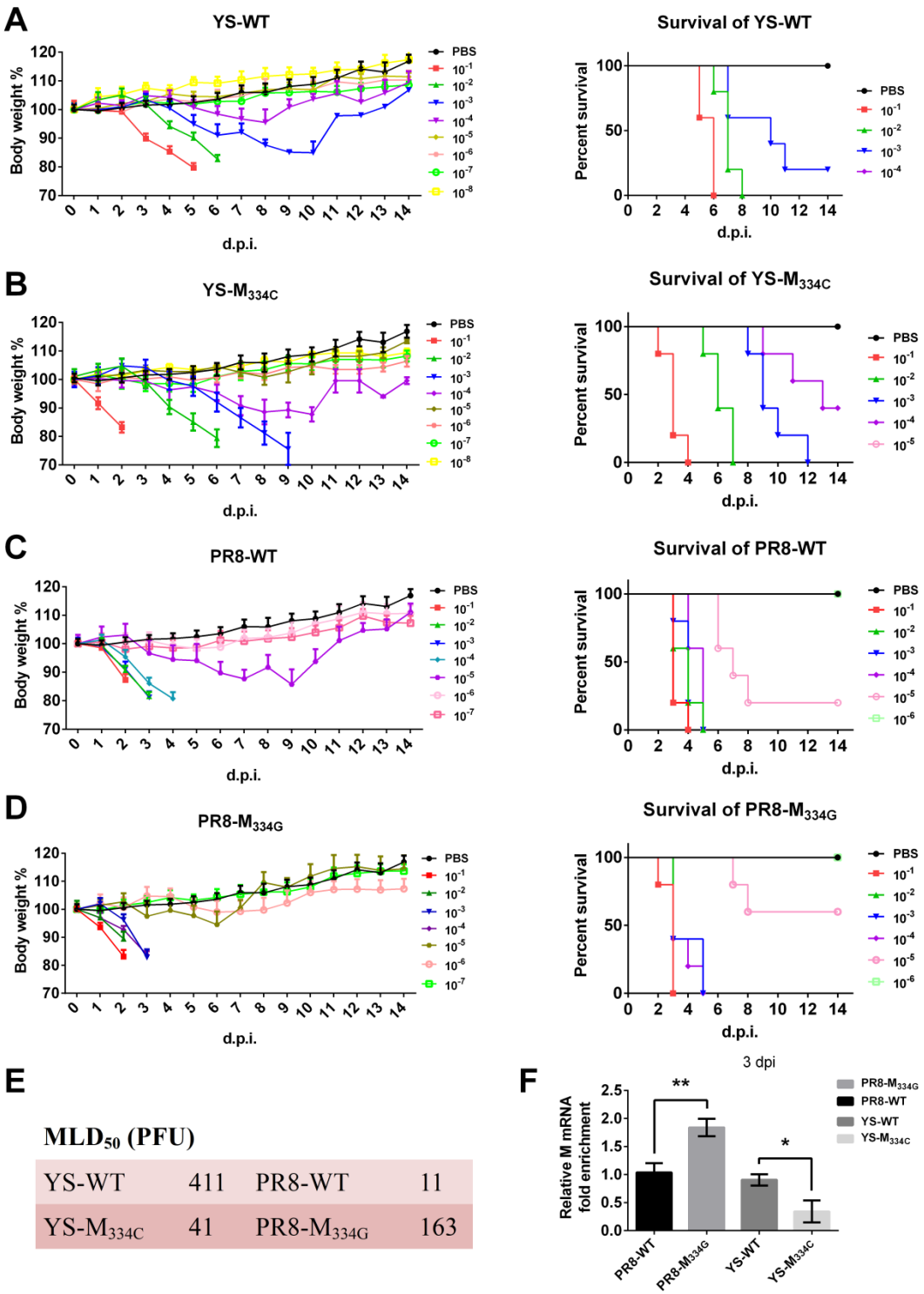

fig. S6. MLD_{50} of the WT and M-334 mutation of PR8 and YS viruses. (A-D) Weight loss of mortality of mice infected with same doses of WT and their mutated viruses. Six-week-old BALB/c mice were intranasally infected with 10-fold serially

diluted (same dose) of wild-type (WT) or their mutants of PR8 or YS. Body weight (left) and survival (right) were monitored daily for two weeks (n=5). **(E)** MLD_{50} of WT and mutant viruses. MLD_{50} was calculated by the Reed and Muench method. (**F**) mRNA in murine lungs were purified with either the control IgG or the TRA2A antibody. The purified RNAs were then analyzed by specific RT-qPCR assay. *, $p < 0.05$; **, $p < 0.01$.

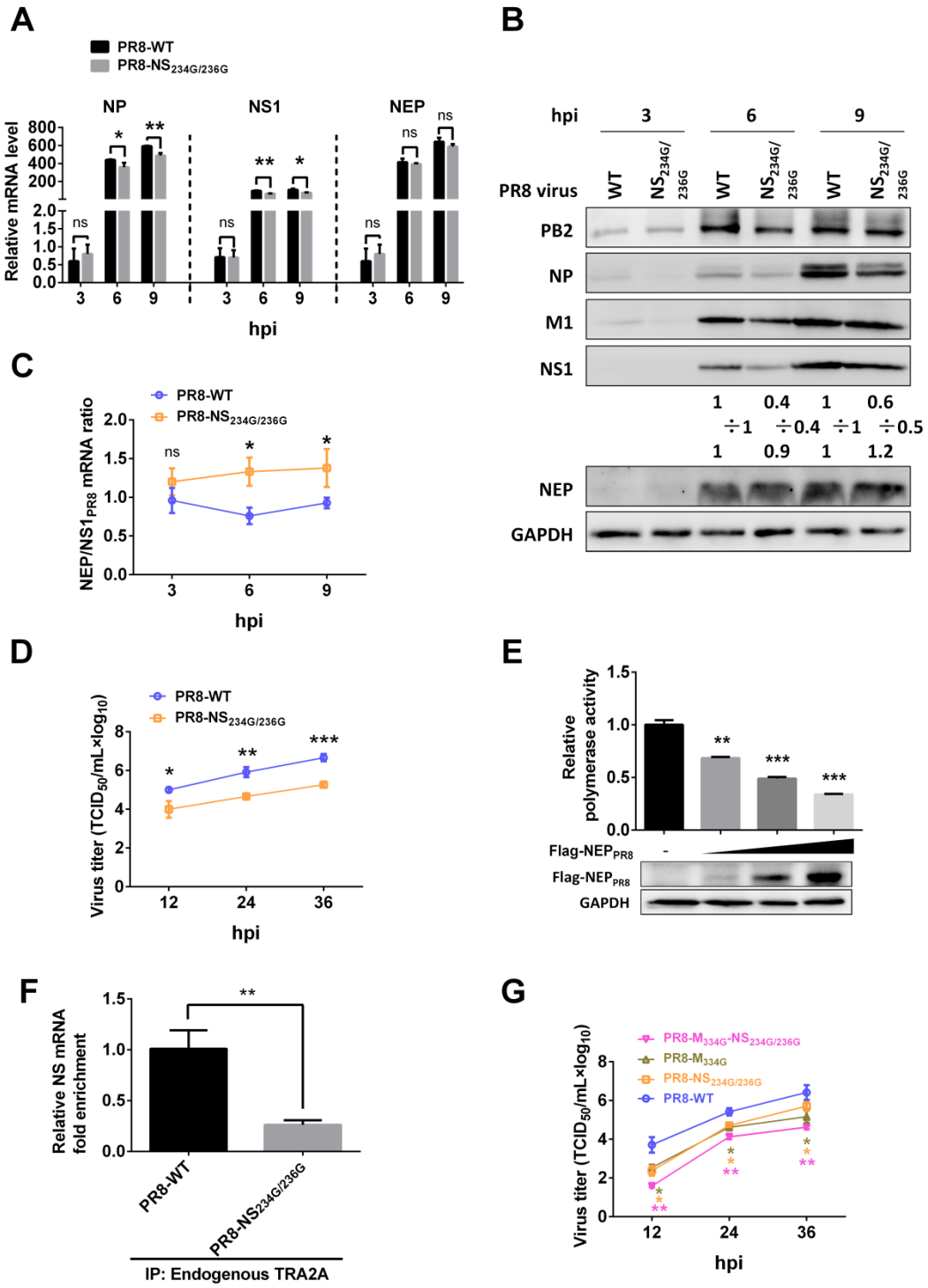

fig. S7. NS-234/236 mutations or double mutations in both M and NS segments alter PR8 virus replication and mRNA splicing. (A-C) A549 cells were infected with the PR8-WT or PR8-$NS_{234G/236G}$ virus, total RNA was isolated and analyzed by RT-qPCR with primers specific to NP, NS1 and NEP mRNAs (A). Cells lysates were

subjected to western blotting analysis using antibodies against respective influenza virus proteins. GAPDH was used as the loading control. Each protein band was quantified by ImageJ and normalized to GAPDH levels (B). Ratios of NEP to NS1 mRNA (C) over time were presented. **(D)** A549 cells were infected with indicated virus at an MOI of 0.01, virus titers were determined by $TCID_{50}$ assay on MDCK cells at indicted time points. **(E)** HEK 293T cells were transfected with indicated plasmids. Cells were lysed at 24 hpi, and firefly luciferase and Renilla luciferase activities were determined with the Dual-Luciferase reporter assay system. **(F)** A549 cells were infected with the wild type or their mutants of PR8 virus, and the mRNA were pulled-down with the control IgG or TRA2A antibody. The RNAs were then analyzed by RT-qPCR to determine the NEP/NS1 ratio. **(G)** A549 cells were infected with indicated viruses at an MOI of 0.01, virus titers were determined by $TCID_{50}$ assay on MDCK cells at indicted time points. Means ± SD (error bars) of three independent experiments are indicated. ns, not significant; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

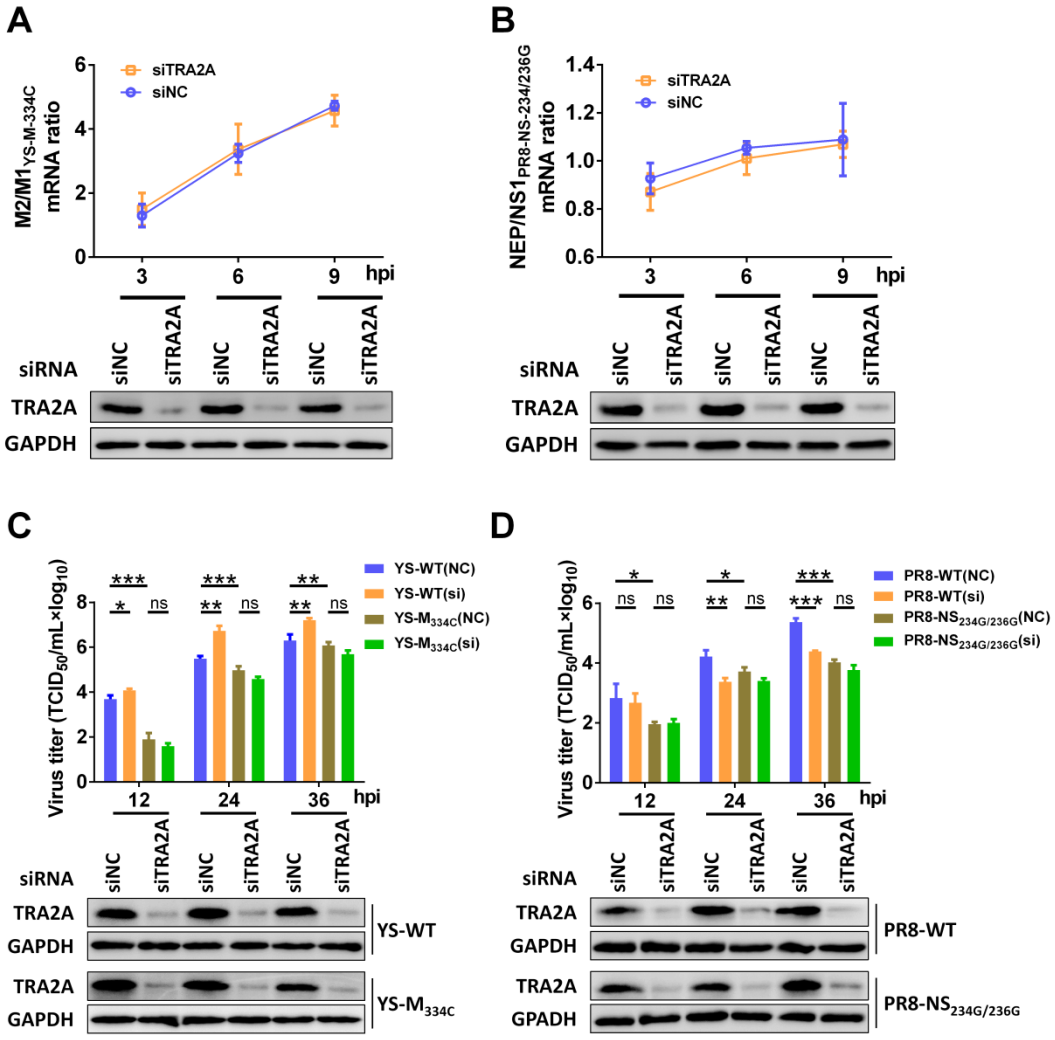

fig. S8. TRA2A does not regulate the ISS mutated mNRA splicing and affects virus replication of ISS mutant viruses. (A, B) A549 cells were transfected with either siNC or siTRA2A for 24 h, and then infected with the YS-M_{334C} (A) or PR8-$NS_{234G/236G}$ (B) virus at an MOI of 5. The total RNAs were measured by RT-qPCR and the NEP/NS1 and M2/M1 mRNA ratios were also calculated. Cell lysates were collected at 3, 6, and 9 hpi, and subjected to western blotting analysis. **(C, D)** A549 cells were transfected with either siNC or siTRA2A for 24 h, and then infected with the indicated virus at MOI of 0.01. Virus titers were determined at 12, 24, and 36 hpi. Cell lysates were collected and subjected to western blotting analysis. Data were represented as means ± SD (error bars) of at least three independent experiments. ns, not significant; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

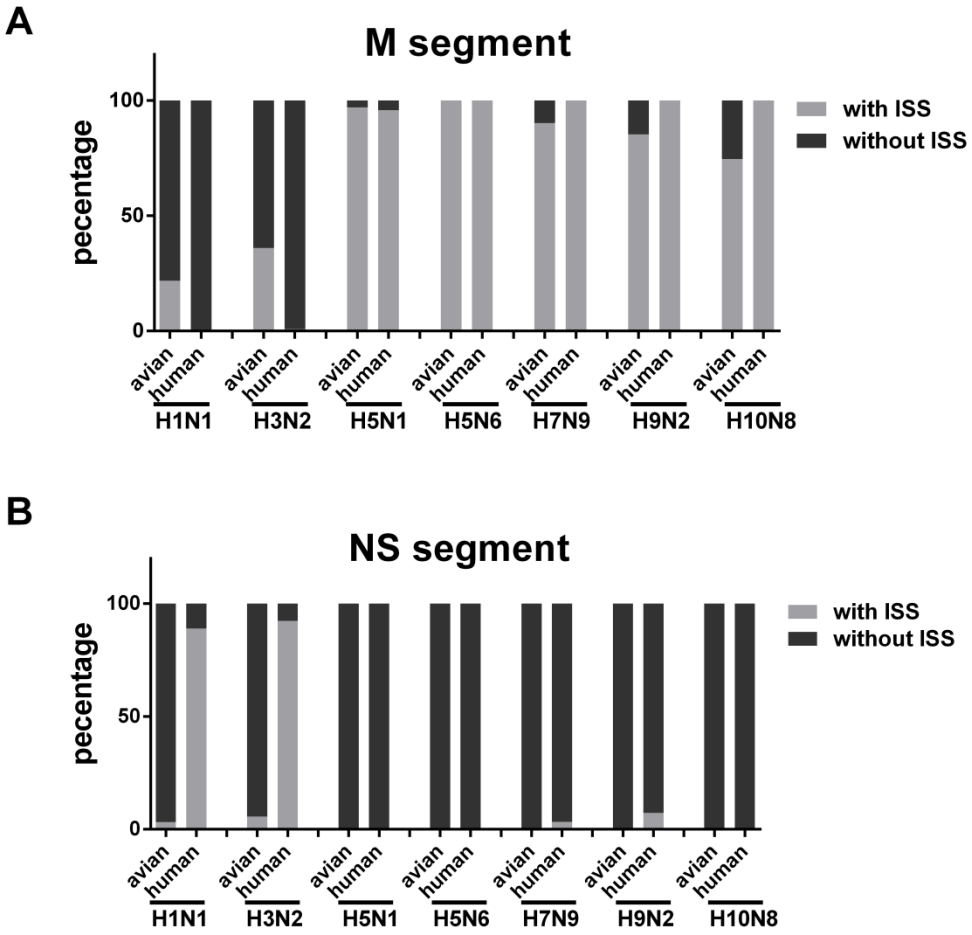

fig. S9. Analysis of ISS in M and NS segments of IAV. (A) The ratio of ISS in M segment (with ISS number/without ISS number/total of numbers analyzed) in each analyzed subtype of IAVs isolated from human and avian species. Both human H1N1 (42/9018/9060), H3N2 (76/8457/8533), H5N1 (162/3/165), H5N6 (3/0/3), H7N9 (80/0/80), H9N2 (13/0/13), and H10N8 (3/0/3), and avian H1N1 (90/321/411), H3N2 (93/165/258), H5N1 (1799/59/1858), H5N6 (213/0/213), H7N9 (305/33/338), H9N2 (1238/212/1450), H10N8 (77/35/102) viruses were analyzed. **(B)** The ratio of ISS in NS segment (with ISS number/without ISS number/total of numbers analyzed) in each analyzed subtype of IAVs isolated from human and avian species. Both human H1N1 (6772/839/7611), H3N2 (7460/619/8079), H5N1 (0/186/186), H5N6 (0/27/27), H7N9 (3/93/96), H9N2 (1/13/14), and H10N8 (0/4/4), and avian H1N1 (14/421/435), H3N2 (16/266/282), H5N1 (0/2002/2002), H5N6 (0/219/219), H7N9 (0/348/348), H9N2 (9/1749/1458), H10N8 (0/73/73) viruses were analyzed.

Table S1. Primers, si-RNA and probes

Primers for qPCR	
Name	Sequence
TRA2A-F	TCTCGTAGTCCATCAAGGGTTTC
TRA2A-R	GCCAGTATGTCTTCTCCGGTTAG
YS-NS1-F	GGAAGAGGCAACACTCTTGG
YS-NS1-R	TTTCTGCTTGGGCATGAGCA
YS-NEP-F	CTGTGTCAAGCTTTCAGGAC
YS-NEP-R	AGTCTCCTATCCTCATCACT
YS-M1-F	CGCGCAGAAACTTGAAGATG
YS-M1-R	TGGATCTCCATTTCCATTTA
YS-M2-F	GCACTTGATATTGTGGATTC
YS-M2-R	CAATTCTATGTTGACAAAATG
YS-NP-F	GCGTCTCAAGGCACCAAAC
YS-NP-R	AGCCTCCCTTCATAGTCAC
YS-PB1-F	TGAAACACTAGCGAGGAG
YS-PB1-R	ATCATTGCCAGAAACATC
PR8-NS1-F	GGAAGGGGCAGTACTCTCGG
PR8-NS1-R	TTTCTGCTTGGGTATGAGCA
PR8-NEP-F	CTGTGTCAAGCTTTCAGGAC
PR8-NEP-R	AGTCTCCCATCCTTATCACT
PR8-M1-F	CGCACAGAGACTTGAAGATG
PR8-M1-R	TGGATCCCCGTTCCCATTAA
PR8-M2-F	GCACTTGACATTGTGGATTC
PR8-M2-R	GCACTTGACATTGTGGATTC
PR8-NP-F	GCGTCTCAAGGCACCAAAC
PR8-NP-R	AACCGTCCCTCATAATCAC
PR8-PB1-F	ACTTACTGGTGGGATGGT
PR8-PB1-R	CTGAAATTGGCAACAAAC

GAPDH-F	GCAAAGGCTGTGGGCAAGG
GAPDH-R	GGAGGAGTGGGTGTCGCTG
U87 scaRNA-F	ATGGGATCATGGAGCAGCTG
U87 scaRNA-R	TCACACCCATGACTGCCACT
Primers for semi-quantitative PCR	
M1/M2-F	TTCTAACCGAGGTCGAAAC
M1-R (400bp)	CCCATCCTGTTGTATATGAG
M2-R (246bp)	CCACAGCACTCTGCTGTTCCT
Primers for amplification	
TRA2A-F	CGGGATCCG ATGAGTGATGTGGAGGAAA
TRA2A-R	CCCAAGCTT ATAGCGTCTTGGGCTGT
pCDNA3-M-F	GCGAATTC GTAATACGACTCACTATAGGG
pCDNA3-M(full)-R	GCCTCGAG AGTAGAAACAAGGTAGTTTTT
pCDNA3-M360-R	GCCTCGAG GCCCCATGGAATGTTAT
pCDNA3-YS-M333-R	GCCTCGAG TAAGCTATATAAGAAGCT
pCDNA3-PR8-M333-R	GCCTCGAG TTAAACTGTATAGGAAGCT
RNA oligonucleotides for RNA pull-down and EMSA	
YS-M-332-351	CUGAAAAGAGAAAUAACAUU
PR8-M-332-351	CUCAAGAGGGAGAUAACAUU
YS-NS-223-242	CUGGAGGAGGAGUCUGAUGA
PR8-NS-223-242	CUGAAAGGAGAAUCCGAUGA
YS-M-332-351(334C)	CUCAAAAGAGAAAUAACAUU
PR8-M-332-351(334G)	CUGAAGAGGGAGAUAACAUU
CU	CUUUCCUCUCCUUUCUCUCC
Si-RNA	
TRA2A-sense(5’-3’)	GCCUCAGUUUGUACACAACTT
TRA2A-antisense(5’-3’)	GUUGUGUACAAACUGAGGCTT