

Supplementary materials

RNA overwriting of Cellular mRNA by Cas13b-directed RNA-dependent RNA Polymerase of Influenza A Virus

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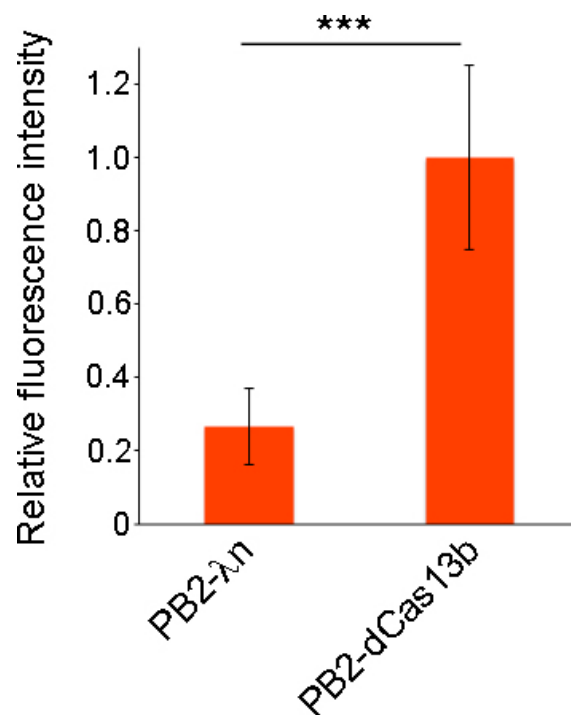


Figure S1. The mScarlet-I fluorescence in RNA overwriting by PB2-λn- or PB2-dCas13b-containing RNP using gRNA 2 and target RNA 3. The graph presents the mean \pm standard deviation from three independent assays ($n = 3$). At 48 hpt, the fluorescence intensity of cell lysates was measured using a microplate reader. Asterisks denote p -values from Welch's t -test (***) $p < 0.001$).

human U6 promoter
5'-GCATACTCCAGCGGATCGATCCAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGAT
TCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACT
GTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAG
TTTGCAGTTTTAAATTATGTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAAGT
ATTTGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGGTAAATCGTGAT
gRNA 2 terminator
GCGACGTCAAGATTGTCGTTGTGGAAGGTCCAGTTTTGAGGGGCTATTACAACTTTTTTT
CTCCGCTGAGCGTACTGAGACGCCGCGGTGGAGCTCCAGCTTTTGTTC-3'

Figure S3 Partial DNA sequence of gRNA 2 expression plasmid (phU6-gRNA 2).

5'-TACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTC
 ACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTGTTTGGCACCAA
 CMV promoter
 ATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGT
 AGGCGTGACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCA
 CTGCTTACTGGCTTATCGAAATTAATACGACTCACTACGGACAATCTTGACGTGCGATCA
 CGATTTACCTCTCGCTCTCAGCACCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGG
 GTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGT
 CCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCA
 CCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCG
 TGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGC
 EGFP
 CATGCCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTAC
 AAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTG
 AAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACT
 ACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAA
 CTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG
 CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCGACAACCACTACCTGAGC
 ACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGG
 AGTTCGTGACCGCCGCGGGATCACTCTCGGCATGGACGAGCTGTACAAGAAGCTTAG
 CCATGGCTTCCCGCCGGCTGTGGCTGCTCAGGATGATGGCACGCTGCCCATGTCTTGT
 PEST
 GCCCAGGAGAGCGGGATGGACCGTCACCCTGCAGCCTGTGCTTCTGCTAGGATCAATG
 TGTAGAATTCACTCGATATCTGCATCTAGAGGGCCCTATTCTATAGTGTACCTAAATG
 CTAGAGCTCGCTGATCAGCCTCGACTGTGCTTCTAGTTGCCAGCCATCTGTTGTTTGC
 BGH polyadenylation signal
 CCCTCCCCCGTGCTTCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAA-3'

Figure S4 Partial DNA sequence of target RNA 3 expression plasmid (pcDNA3-target 3).

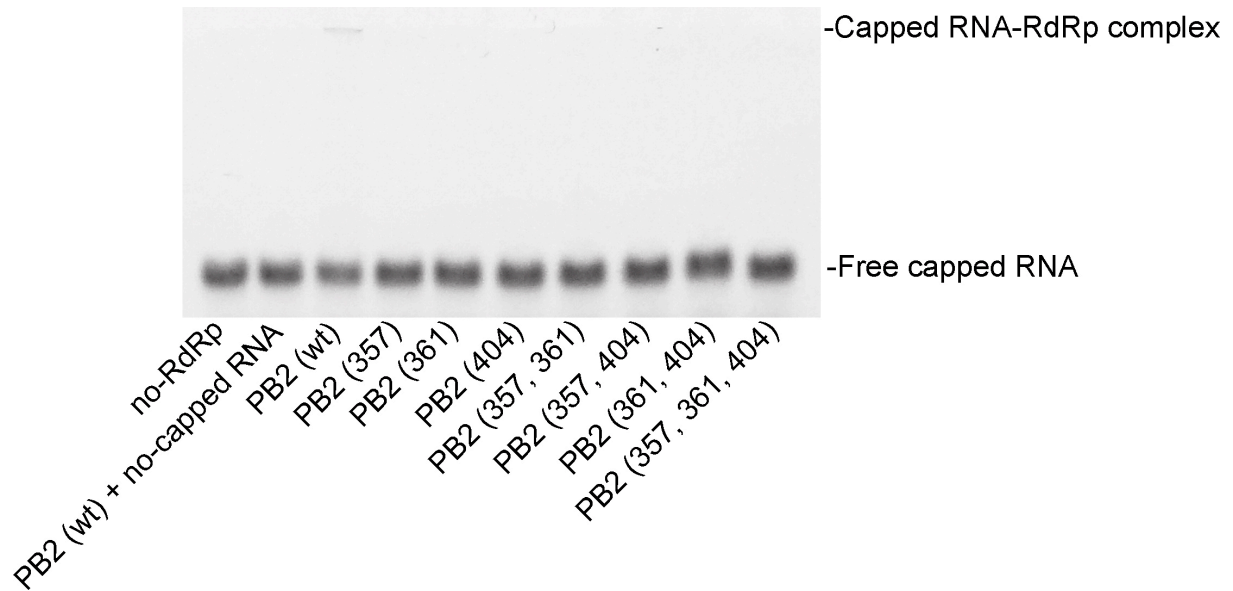


Figure S5 EMSA for interaction between 20-nt capped-RNA and RdRp containing PB2 mutant. EMSA was performed on a 4% native polyacrylamide gel in TBE buffer and run for 40 min with a field of 100 V at room temperature. Subsequently, gel was stained with GelGreen. Before loading, the mixture containing recombinant RdRp and 20-nt capped-RNA were incubated in reaction buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM DTT, 5 mM $MgCl_2$,) for 30 min at room temperature.

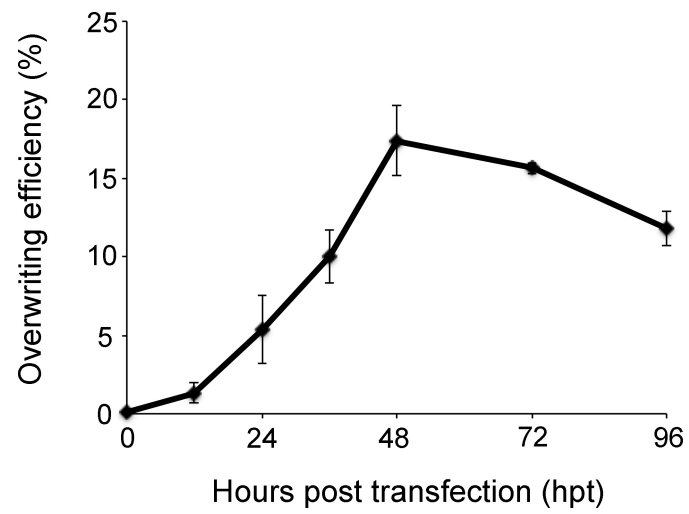


Figure S6 Time-course for RNA overwriting by PB2-dCas13b-containing RNP using gRNA 2 and target RNA 3. Overwriting efficiency was calculated in comparison to the case when RNA overwriting was performed using plasmid for expression of mScarlet-I mRNA instead of target EGFP mRNA.



Figure S7 Sequence of overwriting products. The upper and lower sequences indicate the target and overwritten RNA, respectively. The underline represents the AG site, which is the initiation point of overwriting.

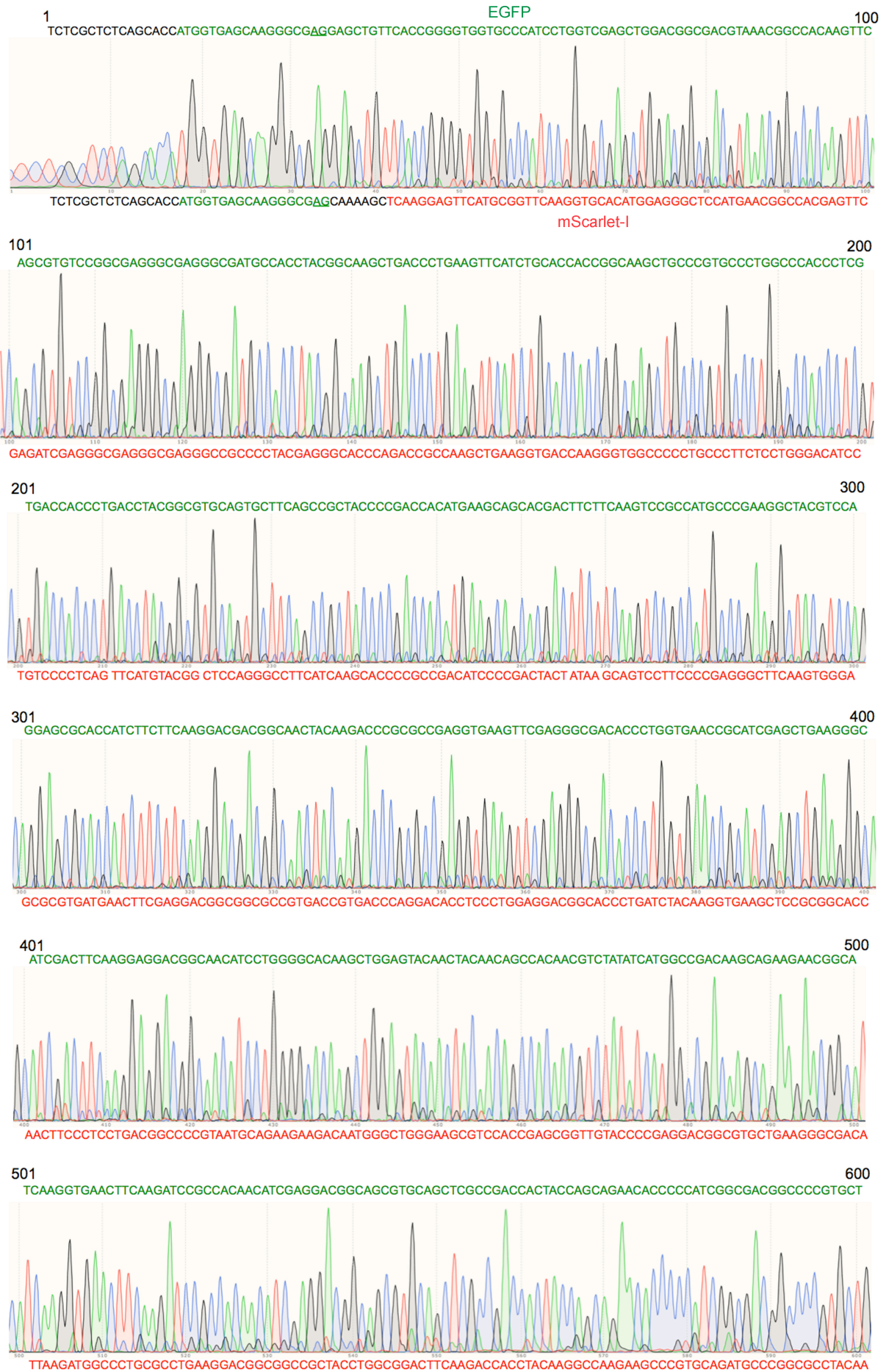


Figure S8 Sequencing data after overwriting in ORF. The upper and lower sequences indicate the target and overwritten RNA, respectively. The underline represents the AG site, which is the initiation point of overwriting.