Electronic Supplementary Material (ESI) for Analyst.
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Supplementary Fig. 1 Schematic diagrams for wild-type PB2 and PB2-KO genes. Genes are illustrated in the positive-strand orientation. In the *PB2-KO* gene, the open reading frame (ORF) for GFP was inserted between 120 nucleotides (nt) of the 5′ and 336 nt of the 3′ coding sequences along with the corresponding sequences from non-coding regions (NCR). The 'atg' triplets in the nucleotide positions at 1 to 3 and 31 to 33 were mutated to 'ctg' to abolish undesirable start codons. Dark gray indicates ORF for PB2. Light gray indicates the ORF for GFP.

Supplementary Fig. 2 Growth capacity of NA-tagged viruses. 10^3 $TCID_{50}$ of PR8NA/His (a), CA07NA/Wt (b), CA07NA/FLAG (c), or CA07NA/His (d) viruses were inoculated onto MDCK-PB2 cells, and the bright field (BF) and fluorescence (GFP) images were periodically obtained at 0, 12, 24, 36, and 48 hpi.

Supplementary Fig. 3 Plaque formation of NA-tagged viruses. Ten-times serially diluted virus solutions were inoculated onto MDCK-PB2 cells seeded on 6-well plates, and then overlaid with MEM containing agar. After 48-h incubation, the cells were fixed and stained with crystal violet solution.

Supplementary Fig. 4 Non-specific signals for anti-peptide-tag antibodies in lectin microarray analyses. Virions were immunoprecipitated from PBS (a; as a negative control of the immunoprecipitation), or viral solutions containing either PR8NA/Wt (b) or CA07NA/Wt (c). Immunoprecipitated antigens were subjected to antibody-overlay lectin microarray with anti-FLAG (closed bars) or anti-His (open bars) antibodies. Data from the gain = 105 were used for the analysis. The data are presented as the mean signal of three spots ± standard deviation (SD).

Supplementary Fig. 5 Glycan profiles of NA from NA-tagged viruses. Glycan profiles of NA from PR8NA/FLAG (a), PR8NA/His (b), CA07NA/FLAG (c), and CA07NA/His (d) viruses were evaluated using a lectin microarray for 45 lectins. Virions were immunoprecipitated from NA-tagged viruses (closed bars), wild-type viruses (gray bars), or PBS (open bars), and immunoprecipitated antigens were subjected to antibody-overlay lectin microarray with the corresponding anti-peptide-tag antibodies. Data from the gain = 105 were used for the analysis. The data are presented as the mean signal of three spots ± standard deviation (SD).

Supplementary Fig. 6 Successive detection of HA on the lectin microarray slide after imaging of the tagged-NA. Raw images obtained for each condition in the lectin microarray are indicated (a–d). The digitized graphs based on image (b) are shown in the closed bar of (e). Virions were immunoprecipitated from PR8NA/FLAG virus (closed bars), PR8NA/Wt virus (gray bars) or PBS (open bars), and immunoprecipitated antigens were subjected to an antibody-overlay lectin microarray with successive detection with anti-FLAG and anti-HA antibodies. Data from the gain = 105 were used for the analysis. The data are presented as the mean signal of three spots ± standard deviation (SD).

Supplementary Fig. 7 Susceptibility of the HA and NA from PR8NA/FLAG virus to PNGase F and Endo H. The viral glycoproteins were subjected to either PNGase F or Endo H digestion and analyzed by western blotting with anti-HA (upper panel) or anti-FLAG (NA; lower panel) antibodies.

Supplementary Fig. 8 Amino acid sequence alignment of PR8NA and CA07NA. N-glycosylation sequons (N-X-S/T) are highlighted in yellow. Conserved amino acids are indicated with asterisks and amino acid deletions are indicated by hyphens.