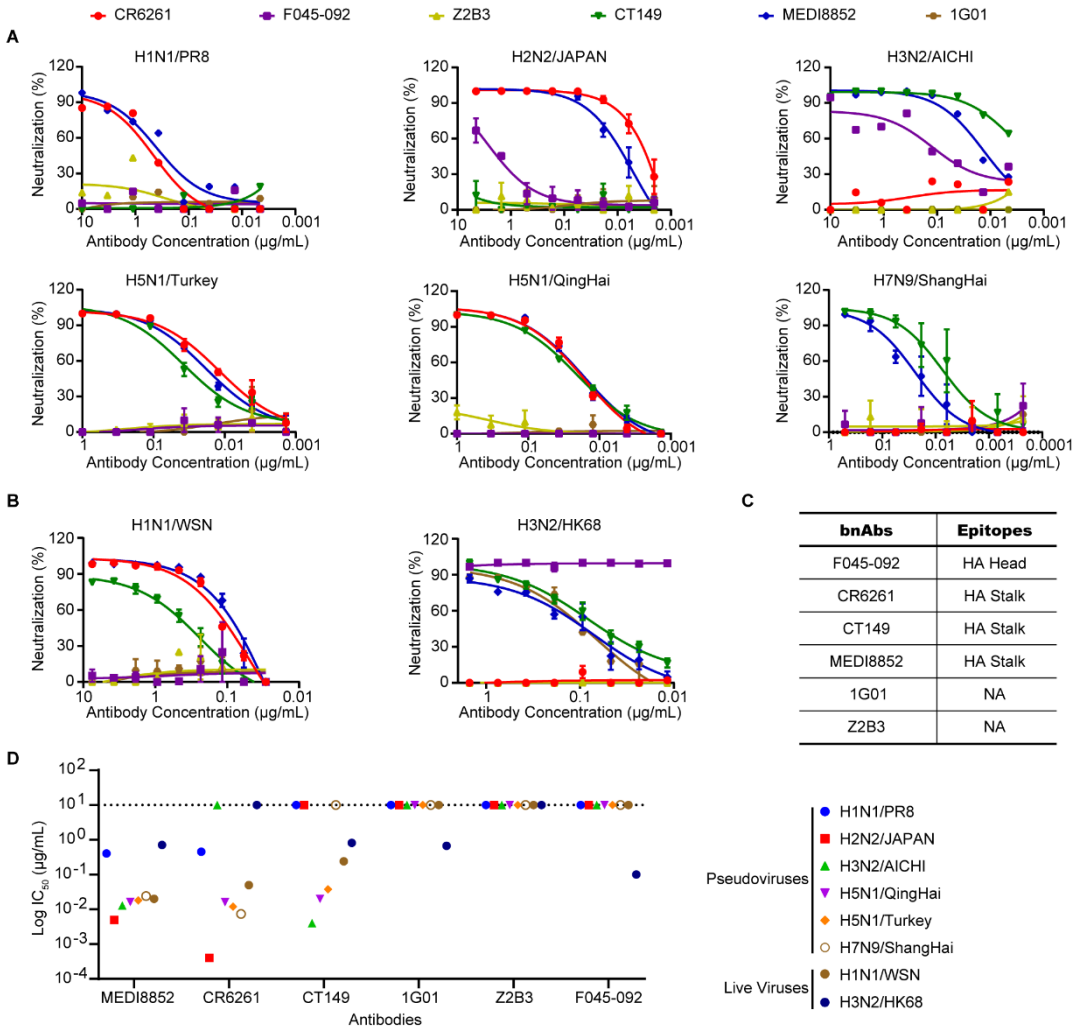

Figure S1. Neutralizing activities of existing bnAbs tested. (A) Neutralizing activity of existing bnAbs against different subtypes of influenza pseudoviruses. Viral infection was determined by measuring luciferase expression. Data represent one of three independent experiments, shown as means ± SEM of technical replicates (n = 3). Source data are provided as a Source Data file. **(B)** Neutralizing activity of existing bnAbs against H1N1/WSN and H3N2/HK68 live viruses. Viral infection levels were determined by measuring NP expression by ELISA. Neutralization percentage was calculated relative to virus-infected cells without antibody treatment. Data represent one of three independent experiments, shown as means ± SEM of technical replicates (n = 3). Source data are provided as a Source Data file. **(C)** Binding epitopes of bnAbs. **(D)** Summary of the IC_{50} values of bnAbs against influenza strains. The dash line indicates the lack of neutralization at the concentration of 10 μg/mL (the IC_{50} of F045-092 against H3N2/HK68 live virus is < 0.1μg/mL).

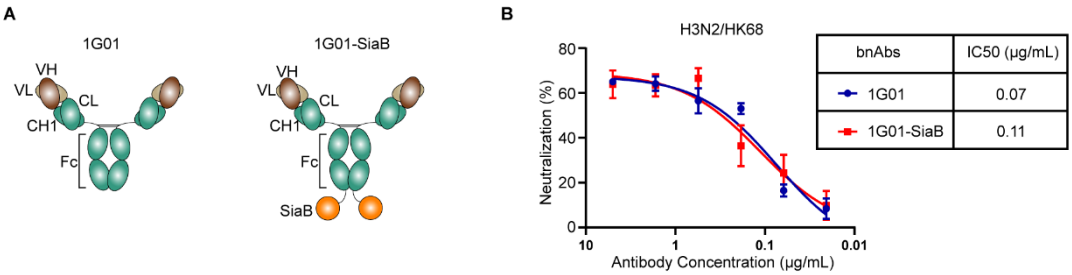

Figure S2. (A) Schematic diagrams of the 1G01 mAb and 1G01-SiaB. **(B)** Neutralizing activity of 1G01 and 1G01-SiaB against live influenza H3N2/HK68 virus. Viral infection levels were determined by measuring NP expression by ELISA. Neutralization percentage was calculated relative to virus-infected cells without antibody treatment. Data represent one of three independent experiments, shown as means ± SEM of technical replicates (n = 3). Source data are provided as a Source Data file.

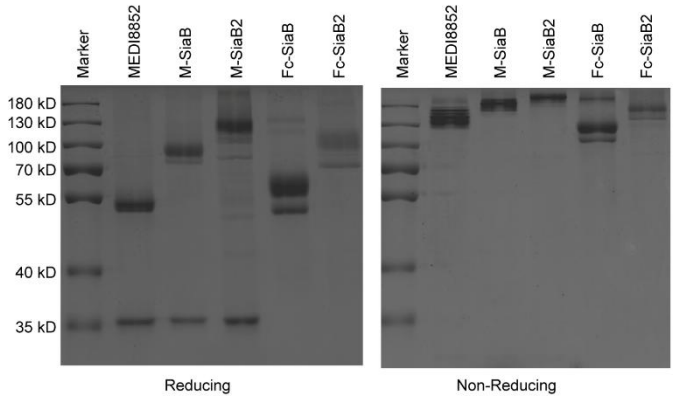

Figure S3. SDS-PAGE analysis of MEDI8852, M-SiaB, M-SiaB2, Fc-SiaB and Fc-SiaB2. Source data are provided as a Source Data file.

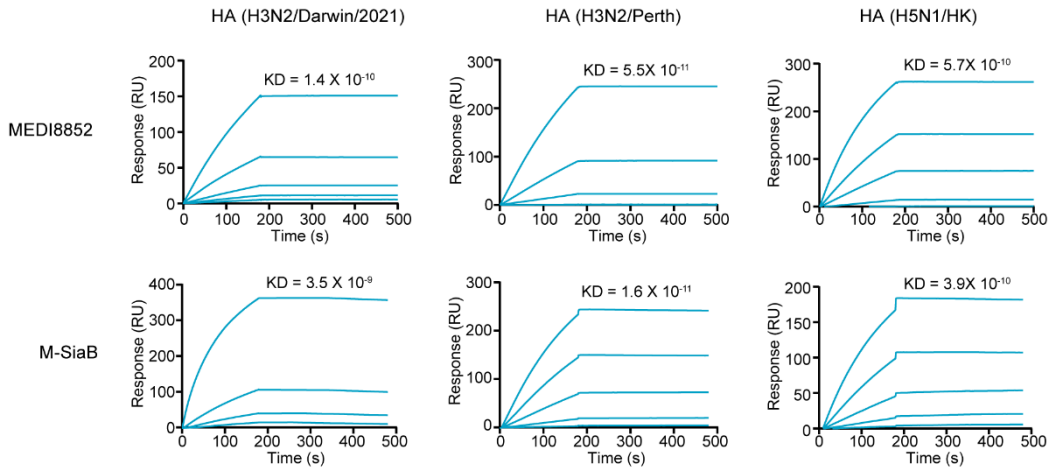

Figure S4. Binding kinetics of MEDI8852 and M-SiaB to HA proteins derived from three different subtypes of influenza viruses by the SPR. The KD values were calculated using a 1:1 binding model by the BIAcore 3000 analysis software.

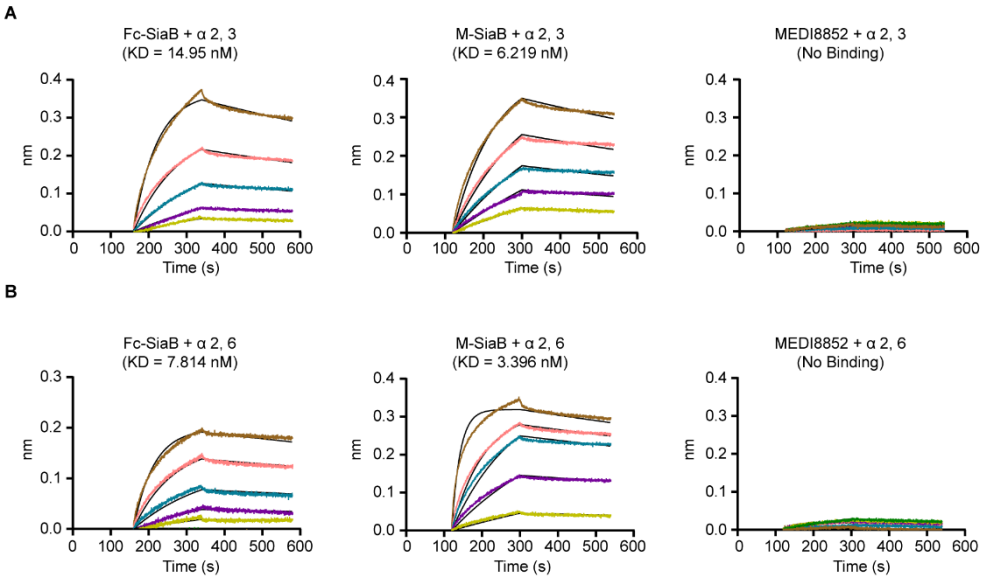

Figure S5. Binding kinetics of Fc-SiaB, M-SiaB, and MEDI8852 to α2,3- (A) and α2,6- (B) linked sialic acid receptors, as measured by BLI. Colored lines represent the original curves, while black lines correspond to the fitted curves. Apparent KD values were determined using a global 1:1 binding model.

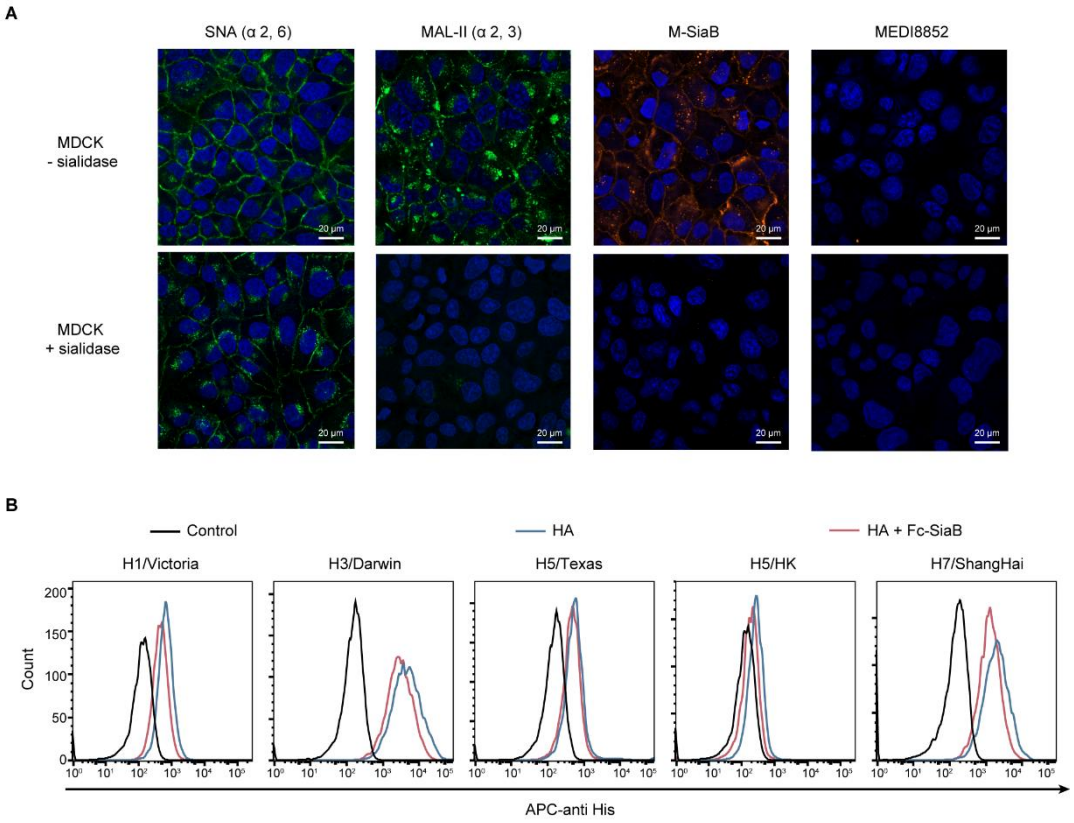

Figure S6. SiaB binds to sialic acid receptors of host cells and blocks HA proteins binding.

(A) Live Image of MDCK cells showing the sialic acid expression and the binding of M-SiaB and MEDI8852 with (Upper Row) and without (Lower Row) the sialidase treatment. α2,6 sialic acid was stained using FITC-conjugated SNA lectin, and α2,3 sialic acid was stained with biotinylated MAL-II lectin followed by FITC-conjugated streptavidin (green). M-SiaB and MEDI8852 were stained using APC-conjugated anti-human IgG antibody (red). Nuclei were stained with DAPI (blue). Scale bars represent 20 μm.

(B) Plots showing the HA proteins binding to MDCK cells with or without Fc-SiaB pre-incubation (in line with Figure 2D data).

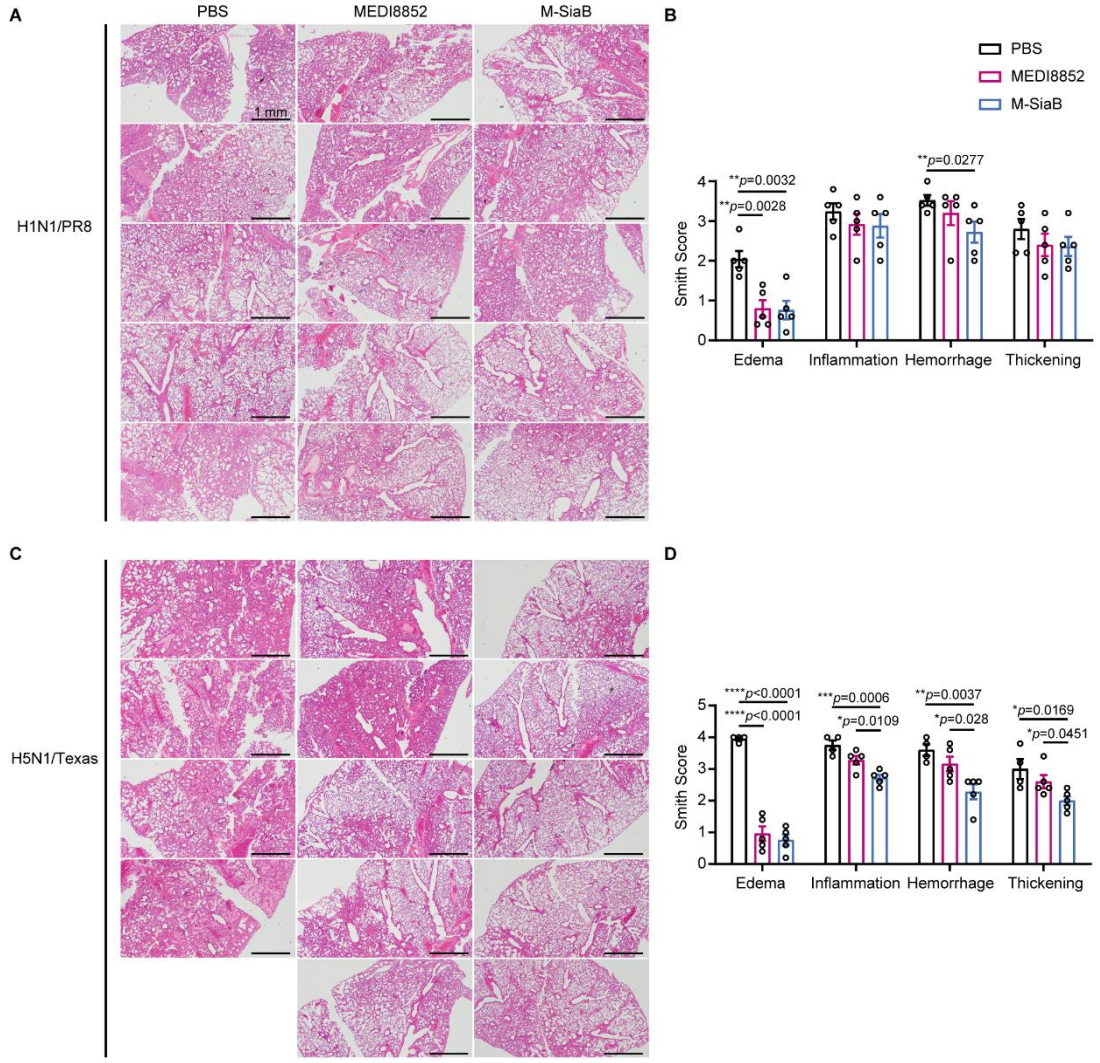

Figure S7. Low magnification images of histopathological analysis of lung tissues and Smith scores.

(A and C) Whole H&E images at 40× magnification of lung tissues from mice pre-treated with PBS or 3 mg/kg MEDI8852 and M-SiaB before infected with (A) H1N1/PR8 and (C) cattle H5N1/Texas/2024. Scale bars represent 1 mm.

(B and D) Smith scores of the lung lesions of inflammatory cell infiltration, edema, alveolar septa thickening and hemorrhage from (B) H1N1/PR8 and (D) cattle H5N1/Texas/2024 infected mice. Data are presented as mean ± SEM from biologically independent mice. Statistical significance was assessed using a two-sided unpaired Student's t-test. Exact P values are shown in the figure. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Source data are provided as a Source Data file.

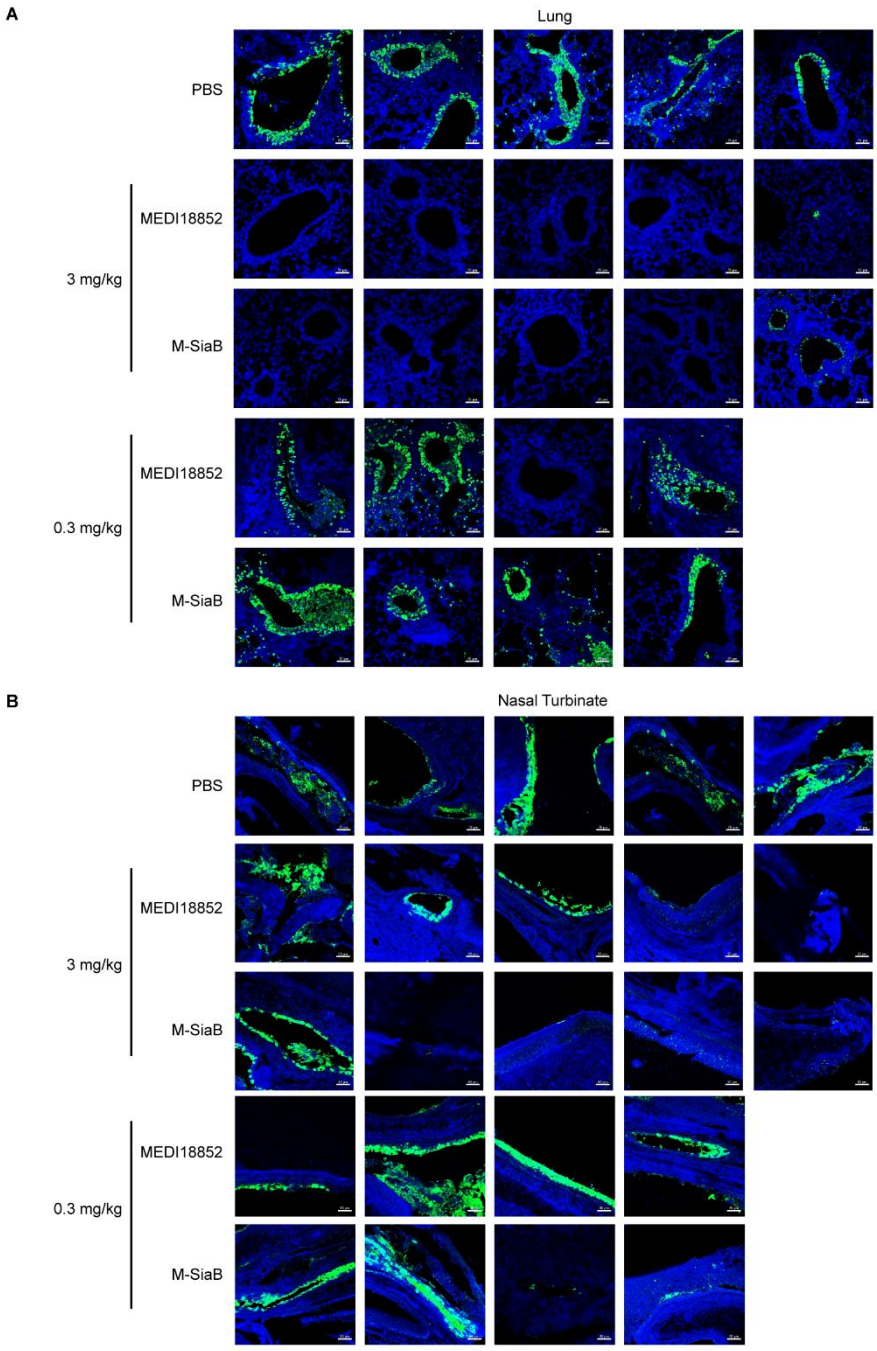

Figure S8. Immunofluorescence detection of influenza NP in lung and nasal turbinate tissues following H1N1/PR8 challenge.

(A) Lung and **(B)** nasal turbinate tissue sections from H1N1/PR8 infected mice pre-treated with PBS, MEDI8852 or M-SiaB were stained with anti-influenza NP antibody followed by GFP-conjugated secondary antibody (green). Nuclei were counterstained with DAPI (blue). Scale bar = 50 μm.

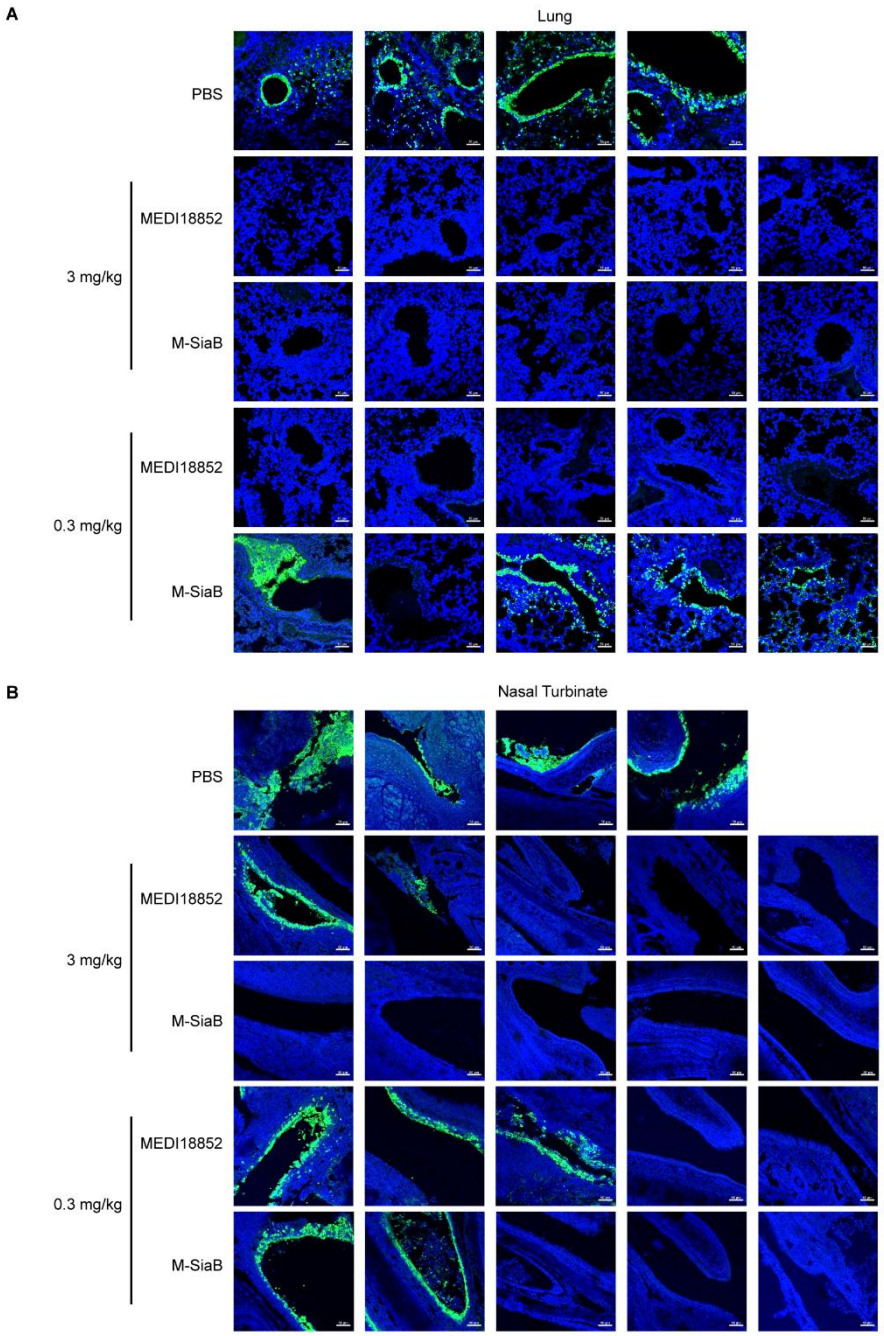

Figure S9. Immunofluorescence detection of influenza NP in lung and nasal turbinate tissues following cattle H5N1/Texas/2024 challenge.
(A) Lung and **(B)** nasal turbinate tissue sections from cattle H5N1/Texas/2024 infected mice pre-treated with PBS, MEDI8852 or M-SiaB were stained with anti-influenza NP antibody followed by GFP-conjugated secondary antibody (green). Nuclei were counterstained with DAPI (blue). Scale bar = 50 μm.

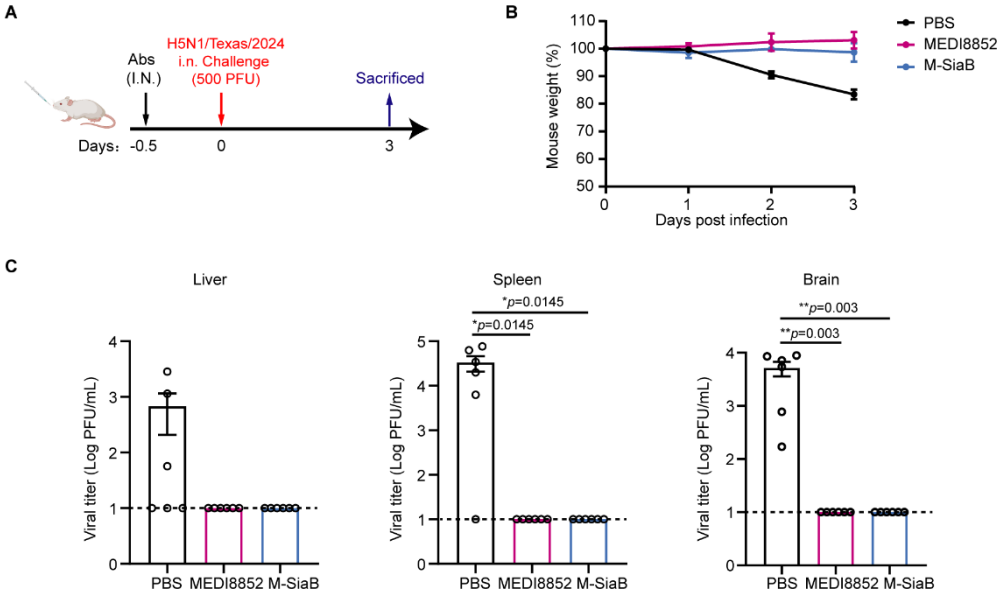

Figure S10. Protection effect of M-SiaB in liver, spleen, and brain tissues in mice following H5N1/Texas/2024 viral challenge.

(A) Experimental timeline. Mice were inoculated intranasally with PBS, MEDI8852 and M-SiaB at doses of 3 mg/kg (n = 6) with PBS as control. 12 hours post-treatment, animals were challenged with 500 PFU cattle H5N1/Texas/2024 and sacrificed at 3 days post-infection for analysis. The mouse cartoon was created in BioRender. CVVT, C. (2026) https://BioRender.com/z92q8sh.

(B) The percentage of daily body weight change after viral infection. Data are presented as mean ± SEM.

(C) Infectious viral titers in liver, spleen and brain homogenates were determined by plaque assay (log10 PFU/mL tissue). Data are presented as mean ± SEM from biologically independent mice. Statistical significance was determined by one-way ANOVA followed by Dunnett's multiple comparisons test using the PBS group as the control. Exact P values are indicated in the figure. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Source data are provided as a Source Data file.

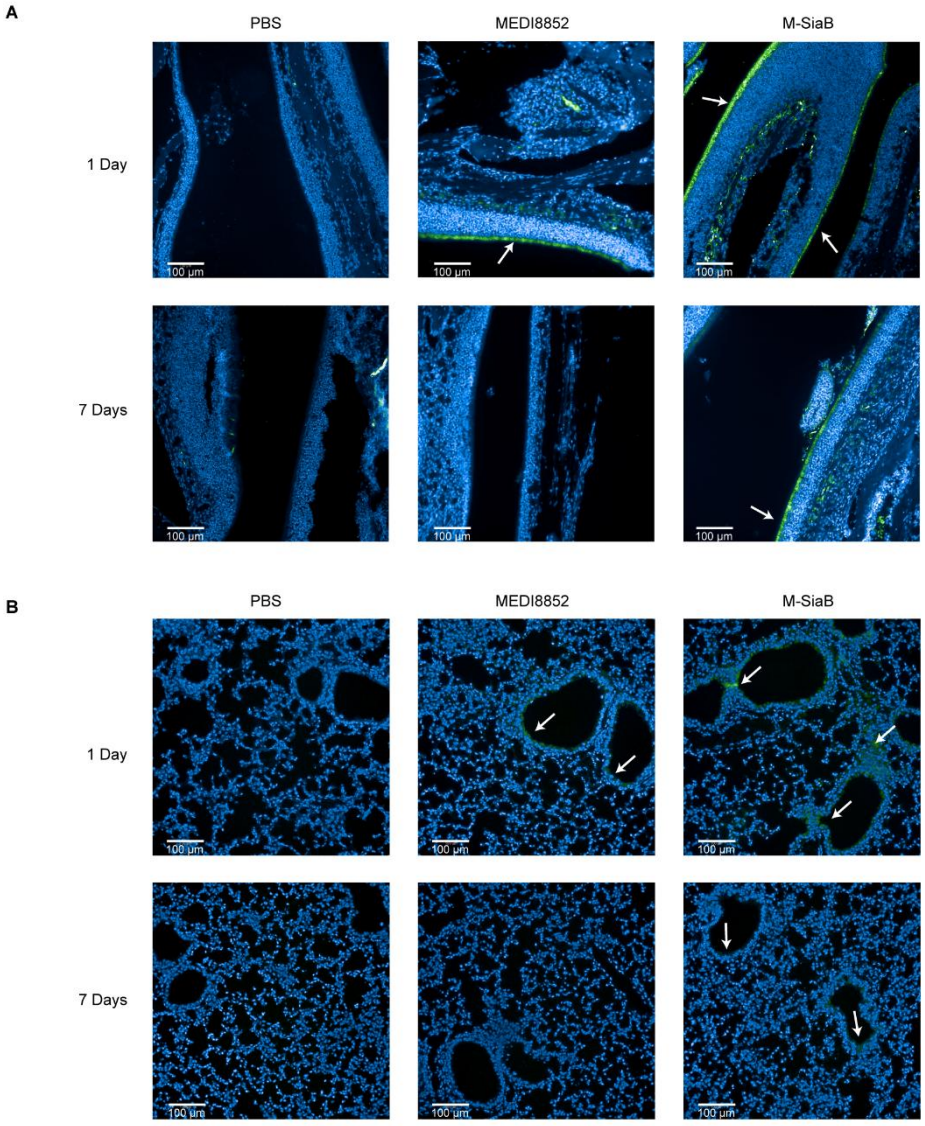

Figure S11. Antibody distribution in nasal turbinate (A) and lung (B) tissues of mice. Nasal turbinate and lung tissues were collected at 1 and 7 days following intranasal administration of a single 100 μg dose of MEDI8852 or M-SiaB. Antibody distribution in the tissues was evaluated by IF staining. MEDI8852 and M-SiaB were detected using Alexa Fluor 488-conjugated goat anti-human IgG (green). Nuclei were counterstained with DAPI (blue). The white arrows indicate bound the antibodies. Scale bar = 100 μm.

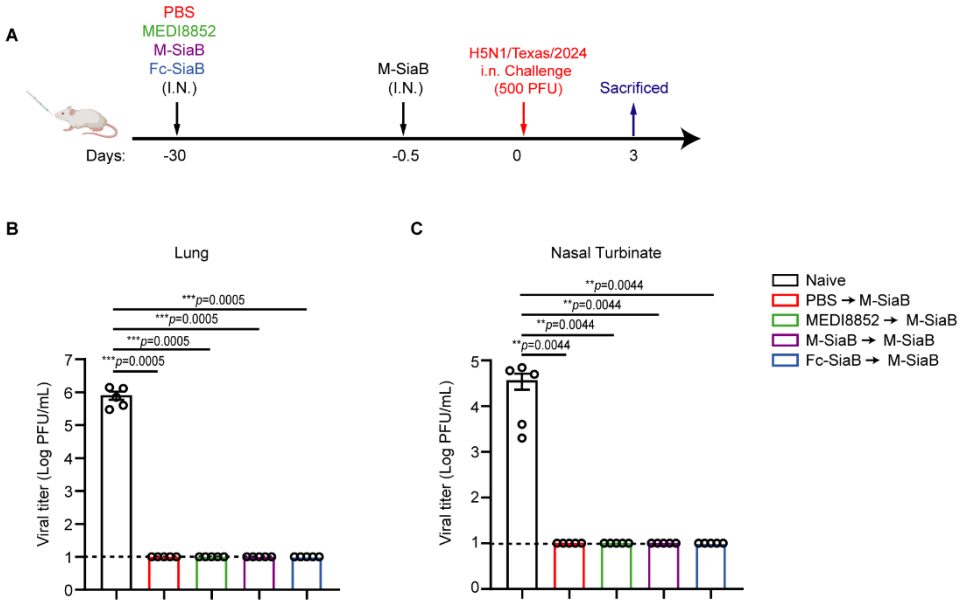

Figure S12. Repeated M-SiaB administration protects against intranasal H5N1 A/Texas/2024 challenge.

(A) Experimental timeline. Mice (n = 5 per group) were intranasally administered with PBS, MEDI8852, M-SiaB, or Fc-SiaB at a dose of 10 mg/kg as the primary immunization. Thirty days later, all groups received 3 mg/kg M-SiaB intranasally. PBS was administered to untreated mice as a control. Twelve hours after the second administration, mice were challenged with 500 PFU of cattle H5N1/Texas/2024 and sacrificed at 3 days post-infection for analysis. The mouse cartoon was created in BioRender. CVVT, C. (2026) https://BioRender.com/z92q8sh.

(B) Infectious viral titers in lung and nasal turbinate homogenates were determined by plaque assay and are presented as $\log_{10}$ PFU/mL of tissue. Data are presented as mean ± SEM from biologically independent mice. Statistical significance was determined by one-way ANOVA followed by Dunnett's multiple comparisons test using the PBS group as the control. Exact P values are indicated in the figure. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Source data are provided as a Source Data file.

Table S1: Viral Strain Designations

	Abbreviation	Isolate Name
Live viruses	H1N1/PR8	A/Puerto Rico/8/1934 (H1N1)
	H1N1/Guangdong/2019	A/Guangdong-Maonan/SWL1536/2019
	H1N1/WSN	A/WSN/1933 (H1N1)
	H3N2/HK/2019	A/Hong Kong/2671/2019 (H3N2)
	H3N2/HK68	A/Hong Kong/1/1968 (H3N2)
	H5N1/Texas/2024	A/dairy cow/Texas/24-008749-003/2024 (2024 cattle H5N1)
Pseudoviruses	H1N1/PR8	A/Puerto Rico/8/1934 (H1N1)
	H2N2/JAPAN	A/Japan/305/1957 (H2N2)
	H3NA/AICHI	A/Aichi/2/1968 (H3N2)
	H5N1/QingHai	A/Bar-headed Goose/Qinghai/1/2005
	H5N1/Turkey	A/turkey/Turkey/1/2005 (H5N1)
	H7N9/ShangHai	A/Shanghai/4664T/2013 (H7N9)

Table S2: Amino acid sequences of antibody constructs used in this study.

Construct	Sequence
MEDI8852 heavy chain	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSYNAVWNWIRQS PSRGLEWLGRTYYRSGWYNDYAESVKSRITINPDTSKNQFSL QLNSVTPEDTAVYYCARSGHITVFGVNVDAFDMWGQGTMV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
MEDI8852 light chain	DIQMTQSPSSLSASVGDRVTITCRTSQSLSSYTHWYQQKPGK APKLLIYAASSRGSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
M-SiaB heavy chain	QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSYNAVWNWIRQS PSRGLEWLGRTYYRSGWYNDYAESVKSRITINPDTSKNQFSL QLNSVTPEDTAVYYCARSGHITVFGVNVDAFDMWGQGTMV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV

	EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGG GGSGGGGSGGGGSALFDYNATGDTEFDSPAKQGWMQDNTN NGSGVLTNADGMPAWLVQGIGGRAQWTYSLSTNQHAQASSF GWRMTTEMKVLSGGMITNYYANGTQRVLPIISLDSSGNLVVE FEGQTGRTVLATGTAATEYHKFELVFLPGSNPSASFYFDGKLI RDNIQPTASKQNMIVWGNGSSNTDGVAAYRDIKFEIQGD
M-SiaB light chain	DIQMTQSPSSLSASVGDRVTITCRTSQSLSSYTHWYQQKPGK APKLLIYAASSRGSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
Fc-SiaB	EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSALF DYNATGDTEFDSPAKQGWMQDNTNNGSGVLTNADGMPAWL VQGIGGRAQWTYSLSTNQHAQASSFGWRMTTEMKVLSGGM ITNYYANGTQRVLPIISLDSSGNLVVEFEGQTGRTVLATGTAAT EYHKFELVFLPGSNPSASFYFDGKLIRDNIQPTASKQNMIVWG NGSSNTDGVAAYRDIKFEIQGD

Table S3: Primer sequences used for RT–qPCR

Primer	Sequence (5'→3')
PR8-NS1-F	TGTCAAGCTTTCAGGTAGATTG
PR8-NS1-R	CTCTTAGGGATTTCTGATCTC
H5N1-NP-F	GAATCCTGGGAATGCTGAAA
H5N1-NP-R	GTTCTGGAGCAGACGGAAAG
β-actin-F	ACGGCCAGGTCATCACTATTG
β-actin-R	CAAGAAGGAAGGCTGGAAAAG

Supplementary uncropped gel