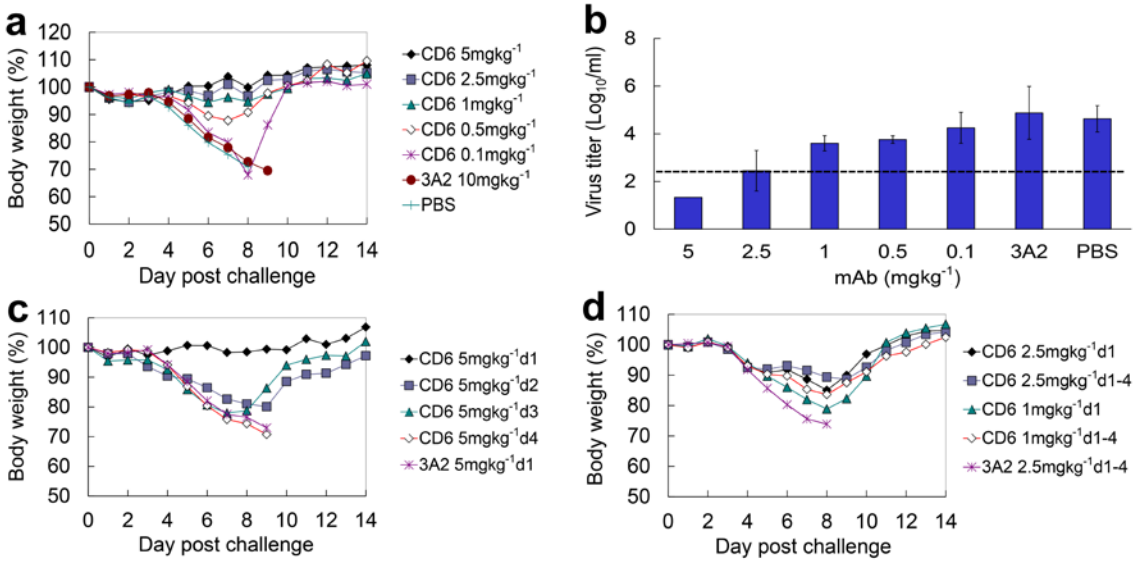
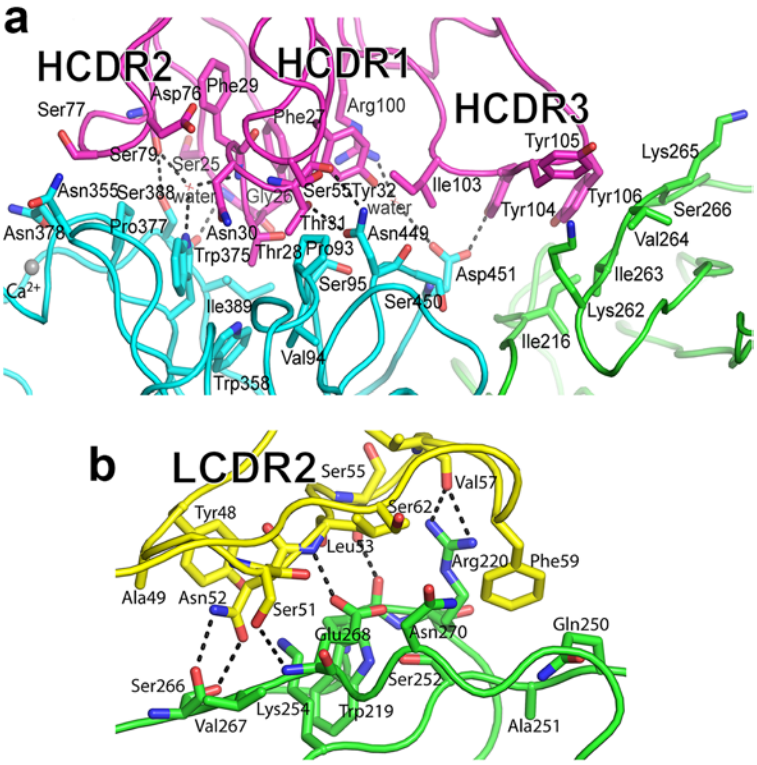
**SUPPLEMENTARY INFORMATION**

# Structural characterization of a protective epitope spanning A(H1N1)pdm09 influenza virus neuraminidase monomers

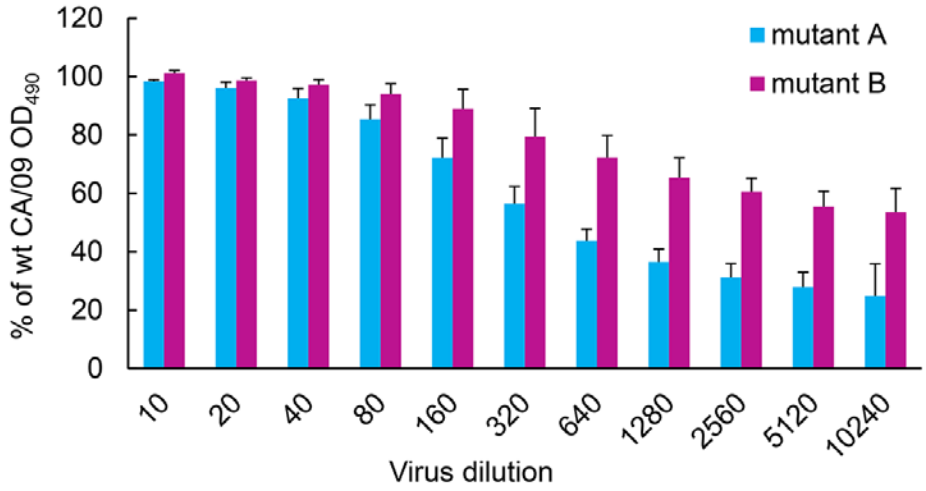
Hongquan Wan, Hua Yang, David A. Shore, Rebecca J. Garten, Laura Couzens, Jin Gao, Lianlian Jiang, Paul J. Carney, Julie Villanueva, James Stevens, Maryna C. Eichelberger



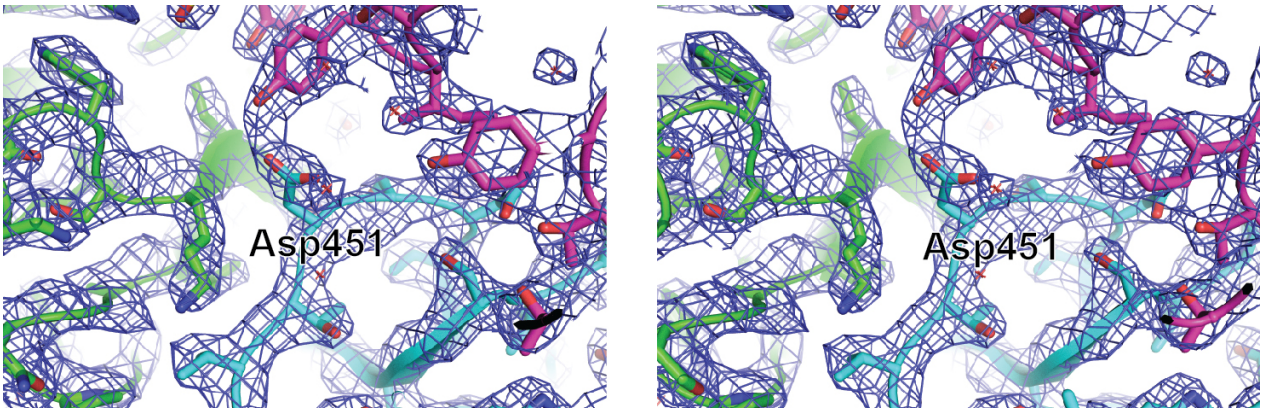

**Supplementary Figure 1: Clinical and virologic evidence of CD6 efficacy in prophylactic and therapeutic studies.** (**a**) Weight loss of mice (n=10) in prophylactic studies. Female DBA/2 mice were injected i.p. with mAb CD6 12 h before challenge with 10 $LD_{50}$ of CA/09-X179A. The survival curves are shown in **Figure 2a** of the main text. (**b**) Lung virus titers of mice (n=5) in prophylactic studies. Mice were treated with CD6 and infected as described in (**a**) and then euthanized on day 3 p.c. The lungs were collected and homogenized for virus titration in MDCK cells. The dotted line denotes the detection limit (2.2 $Log_{10}TCID_{50}$ $ml^{-1}$). A titer of 1.7 $Log_{10}TCID_{50}$ $ml^{-1}$ was arbitrarily set to represent titers below the detection limit. (**c**) Weight loss of mice (n=10) in single dose therapeutic studies. Female DBA/2 mice were infected intranasally with 10 $LD_{50}$ of CA/09-X179A and 5 mg $kg^{-1}$ of mAb CD6 was administered i.p. 1, 2, 3 or 4 days later. (**d**) Weight loss of mice (n=10) treated therapeutically with single or multiple doses of CD6. As described for (**c**), DBA/2 mice were infected with CA/09-X179A and treated with either 2.5 or 1 mg $kg^{-1}$ CD6 24 h later (d1) only or once daily (24 h intervals) for 4 days (d1-4). The survival curves for these experiments are shown in **Figures 2b and 2c** of the main text.



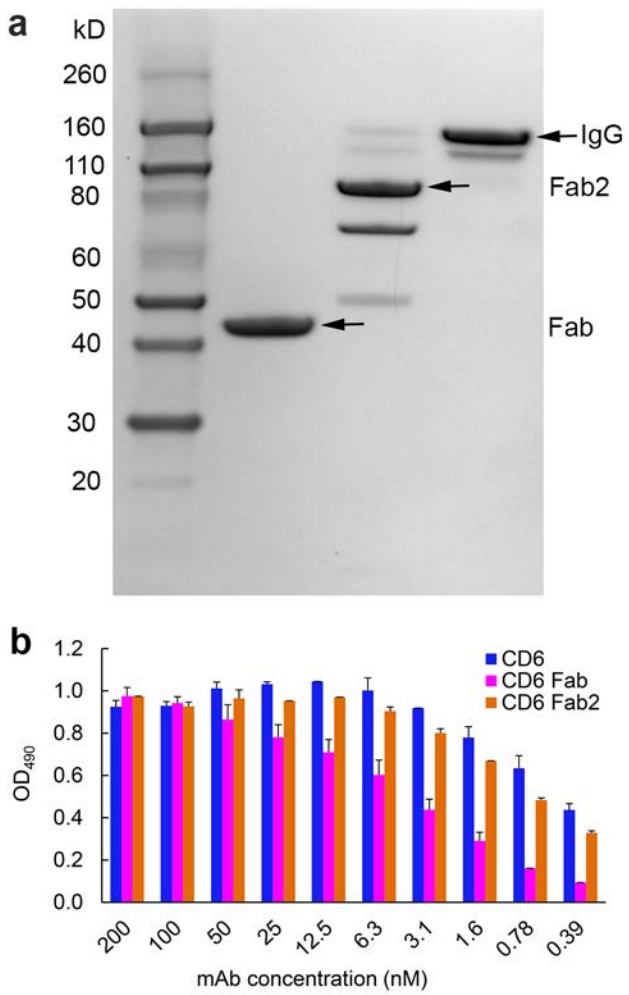

**Supplementary Figure 2: Detailed analysis of the CD6 antibody interface with the NA. (a)** The interface of H chain and CA/09 NA dimer. Waters that mediate interactions at the interface are highlighted as red crosses. (**b**) The interface of LCDR2 and the CA/09 NA monomer. Residues key to the antibody association are highlighted in sticks and hydrogen bonds are shown as dashed lines. Interactions were analyzed using MONSTER[1], PISA[2] and HBPLUS[3].



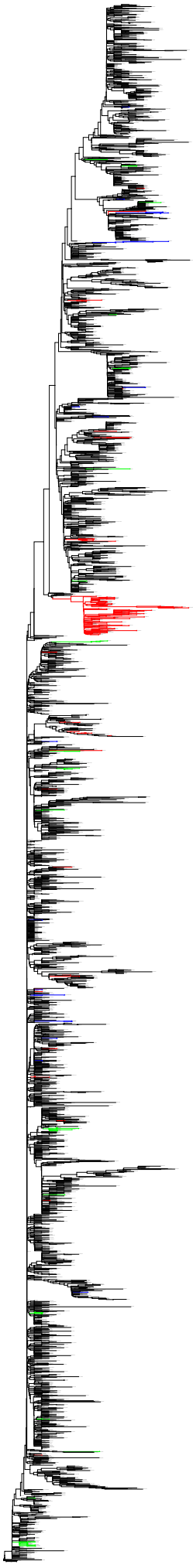

**Supplementary Figure 3: The NAs of escape mutants selected by mAb CD6 are less efficient than wt CA/09 NA.** Enzyme activity was measured with fetuin (MW: 49 kD) as substrate in ELLA, as described in Methods. Briefly, two representative escape mutants (mutants A and B, see the legend to **Figure 5**) and virus containing wt CA/09 NA, were normalized by hemagglutination assay to have the same titer (1024), then serially diluted and added to 96-well plates coated with fetuin, followed by incubation overnight at 37 °C; the plates were washed and then incubated with peanut agglutinin conjugated to horse radish peroxidase to detect the exposed galactose due to the removal of sialic acid from fetuin by NA; the signal was developed using o-phenylenediamine dihydrochloride (OPD) as substrate. The OD values generated with mutants were expressed relative to those obtained at the same dilution as wt CA/09 NA. Data are shown as mean ± s.d. of three independent experiments.



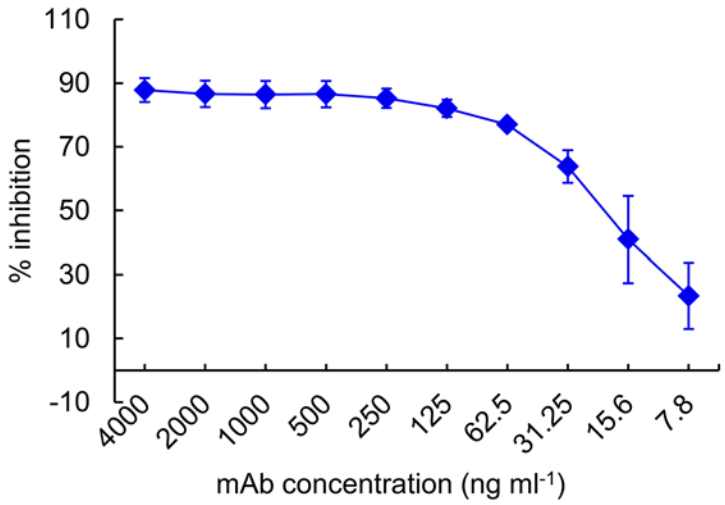

**Supplementary Figure 4: Stereo view of the CD6 antibody interface (pink) with Asp451 of the CA/09 NA dimer (cyan and green).** The 2Fo−Fc electron density map contoured at 1.0 σ is shown in blue.




**Supplementary Figure 5: CD6 Fab and Fab2 retain ability to bind NA.** (**a**) SDS-PAGE of CD6 Fab (lane 2), Fab2 (lane 3) and whole IgG (lane 4). Lane 1: Novex sharp pre-stained protein standard (Invitrogen, Carlsbad, CA, USA; catalog number: LC5800). Lanes 2-4: CD6 Fab, CD6 Fab2 and whole IgG. (**b**) Binding of CD6 Fab, Fab2 and the whole IgG to CA/09 NA measured in ELISA. HRP conjugated goat-anti-mouse IgG (Fab specific) (Sigma-Aldrich, St. Louis, MO, USA; catalog number: A2304) was used to detect the bound antibody. Each bar represents mean + s.d. of two independent assays performed in duplicate.



**Supplementary Figure 6: Phylogenetic tree of pH1N1 NAs.** The tree was constructed with n=7958 pH1N1 NA gene sequences available from the GISAID Epiflu database (www.gisaid.org). Since the phylogenetic tree includes analysis of a large number of NA sequences, color annotation was used to point out the relatively few viruses carrying amino acid changes in NA that may contribute to CD6 binding; a magnification 3200-fold allows the annotation and name of each strain to be read. Amino acid changes within the CD6 epitope are annotated on the phylogenetic tree as follows: NA sequences that have changes at residue 95 are shown as green diamonds; changes at residue 449 are shown as blue triangles; changes at residue 451 which do not have D451G in the sequence are shown as open red circles; changes of D451G alone are shown as closed red circles; and changes that include D451G and N386S are shown as closed red squares.




**Supplementary Figure 7: Inhibition of the NA activity of A/Bethesda/NIH107-D31/2009 virus by antibody CD6.** The pH1N1 virus has a H275Y mutation in the NA, and therefore is resistant to NA inhibitors oseltamivir and peramivir. The inhibition was measured with ELLA, in which fetuin (MW: 49 kDa) was used as substrate. Serial dilutions of antibody CD6 were added to wells prior to the addition of virus. Data are shown as mean ± s.d. of three independent experiments.

**Supplementary Table 1 Contacts between buried surface residues of CD6 and CA/09 NA**

| NA monomer A | NA monomer B | Type of contact | Light chain | Heavy chain |
|---|---|---|---|---|
| Pro93 | | Hydrophobic<br>Hydrophobic | | Thr31<br>Ser55 |
| Val94 | | Hydrophobic<br>Water<br>Hydrophobic | | Thr28<br>Asn30<br>Thr31 |
| Ser95 | | Hydrophobic | | Thr28,Thr31,Tyr32 |
| | Ile216 | Hydrophobic | | Tyr106 |
| | Lys217 | Water | | Tyr104 |
| | Trp219 | Hydrogen bond | Ser55 | |
| | Arg220 | Hydrogen bond<br>Hydrophobic | Val57<br>Phe59 | |
| | Asn221 | Hydrogen bond | Ser55 | |
| | Gln250 | Hydrophobic | Phe59 | |
| | Ala251 | Hydrophobic | Phe59 | |
| | Ser252 | Hydrophobic | Phe59 | |
| | Lys254 | Water<br>Hydrophobic | Tyr48<br>Asn52 | Tyr104 |
| | Lys262 | Hydrophobic | | Tyr105 |
| | Ile263 | Hydrophobic | | Tyr105/Tyr106 |
| | Val264 | Hydrophobic | | Tyr105/Tyr106 |
| | Lys265 | Hydrophobic | | Tyr106 |
| | Ser266 | Hydrophobic<br>Water<br>Hydrogen | Ala49<br>Asn52 | Tyr106 |
| | Val267 | Hydrophobic | Ser51 | |
| | Glu268 | Hydrogen<br>Hydrophobic<br>Hydrogen | Ser51<br>Asn52<br>Leu53 | |
| | Asn270 | Hydrophobic | Ser62 | |
| Asn355 | | Hydrophobic | | Ser77 |
| Trp358 | | Hydrophobic | | Thr28 |
| Trp375 | | Water<br>Water<br>Hydrophobic<br>Hydrophobic<br>Hydrophobic | | Phe29<br>Ser79<br>Phe27<br>Thr28<br>Asn30 |
| Pro377 | | Hydrophobic<br>Hydrophobic | | Ser77<br>Asp76 |
| Asn378 | | Hydrophobic | | Ser77 |
| Ser388 | | Hydrogen<br>Hydrogen<br>Hydrophobic<br>Hydrophobic<br>Hydrophobic | | Gly26<br>Ser79<br>Ser25<br>Gly26<br>Phe27 |
| Ile389 | | Hydrophobic<br>Hydrophobic<br>Hydrophobic | | Gly26<br>Phe27<br>Thr28 |
| Asn449 | | H bond<br>Hydrophobic<br>Hydrophobic | | Thr31<br>Thr31<br>Ile103 |
| Ser450 | | Hydrophobic | | Ile103 |
| Asp451[a] | | H bond<br>Water<br>Hydrophobic | | Tyr104<br>Arg100<br>Tyr104 |

[a]Asp451 forms an H-bond with Thr215 of the neighboring NA monomer.

**Supplementary Table 2 Comparison of the 30 residues in the CD6 epitope to those in the NA of BR/07 and VN/04 viruses**

| Virus | Position/residue | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 93 | 94 | 95 | 216 | 217 | 219 | 220 | 221 | 250 | 251 | 252 | 254 | 262 | 263 | 264 |
| CA/09 | P | V | S | I | K | W | R | N | Q | A | S | K | K | I | V |
| BR/07 | S | I | .[a] | . | . | . | K | K | A | . | . | . | . | V | T |
| VN/04 | . | I | N | . | . | . | . | . | . | . | . | . | . | V | . |

**Supplementary Table 2-continued**

| Virus | Position/residue | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 265 | 266 | 267 | 268 | 270 | 355 | 358 | 375 | 377 | 378 | 388 | 389 | 449 | 450 | 451 |
| CA/09 | K | S | V | E | N | N | W | W | P | N | S | I | N | S | D |
| BR/07 | . | . | I | . | . | . | . | . | . | . | . | V | . | . | . |
| VN/04 | . | . | . | . | D | . | . | . | . | . | . | V | . | . | . |

[a]Residues identical to those in CA/09 NA are shown as dots.

**Supplementary Table 3 Variation of CD6 epitope residues in pH1N1 viruses**

| Residue | Mutation rate by year % (number of pH1N1 isolates with mutation)[a] | | | | | Total |
|---|---|---|---|---|---|---|
| | 2009 | 2010 | 2011 | 2012 | 2013 | |
| Pro93 | 0.05 (2) | 0.5 (6) | 0.84 (9) | 0.16 (1) | 1.22 (10) | 0.35 (28) |
| Val94 | 0.02 (1) | 0.08 (1) | 0.09 (1) | 0.33 (2) | 0.24 (2) | 0.09 (7) |
| **Ser95**[b] | 2.13 (91) | 0.67 (8) | 0.28 (3) | 0.66 (4) | 0.37 (3) | 1.37 (109) |
| Ile216 | 0.16 (7) | | | 0.33 (2) | 0.24 (2) | 0.14 (11) |
| Lys217 | 0.07 (3) | 0.17 (2) | | 0.82 (5) | 0.12 (1) | 0.14 (11) |
| Trp219 | | | 0.09 (1) | | | 0.01 (1) |
| Arg220 | 0.23 (10) | 0.67 (8) | 1.49 (16) | 1.48 (9) | 8.19 (67) | 1.39 (111) |
| Asn221 | 0.07 (3) | 0.25 (3) | | 0.66 (4) | 0.24 (2) | 0.15 (12) |
| Gln250 | | 0.08 (1) | | | | 0.01 (1) |
| Ala251 | 0.02 (1) | | | | | 0.01 (1) |
| Ser252 | | | | | | |
| Lys254 | | 0.08 (1) | | | | 0.01 (1) |
| Lys262 | 0.09 (4) | 0.08 (1) | 0.28 (3) | | 0.12 (1) | 0.11 (9) |
| Ile263 | 0.28 (12) | 0.76 (9) | 0.19 (2) | 0.33 (2) | 0.12 (1) | 0.33 (26) |
| Val264 | 0.23 (10) | 2.27 (27) | 2.52 (27) | 0.99 (6) | 1.34 (11) | 1.03 (82) |
| Lys265 | 0.02 (1) | 0.08 (1) | 0.09 (1) | | 0.24 (2) | 0.06 (5) |
| Ser266 | | | | | 0.12 (1) | 0.01 (1) |
| Val267 | 0.07 (3) | 0.08 (1) | 0.28 (3) | 0.66 (4) | | 0.14 (11) |
| Glu268 | 0.02 (1) | 0.08 (1) | | | | 0.03 (2) |
| Asn270 | 0.16 (7) | 0.25 (3) | 0.37 (4) | 0.33 (2) | 0.49 (4) | 0.25 (20) |
| Asn355 | 0.07 (3) | | 0.28 (3) | 0.16 (1) | 0.12 (1) | 0.10 (8) |
| Trp358 | | 0.08 (1) | | | 0.12 (1) | 0.03 (2) |
| Trp375 | | | | | | |
| Pro377 | | 0.08 (1) | 0.09 (1) | | 0.12 (1) | 0.04 (3) |
| Asn378 | | | 0.37 (4) | | 0.12 (1) | 0.06 (5) |
| Ser388 | 0.26 (11) | 0.34 (4) | 0.09 (1) | | | 0.20 (16) |
| Ile389 | 0.23 (10) | 1.17 (14) | 4.19 (45) | 3.79 (23) | 3.18 (26) | 1.48 (118) |
| **Asn449** | 0.14 (6) | 0.25 (3) | 0.19 (2) | 1.15 (7) | 2.44 (20) | 0.48 (38) |
| Ser450 | 0.07 (3) | 0.25 (3) | 0.28 (3) | 0.16 (1) | 2.44 (20) | 0.15 (12) |
| **Asp451** | 0.26 (11) | 1.34 (16) | 2.61 (28) | 18.78 (114) | 3.18 (26) | 2.46 (196) |
| Total | 4268 | 1192 | 1073 | 607 | 818 | 7958 |

[a]Data were generated with 7958 pH1N1 NA sequences available from GISAID (www.gisaid.org).

[b]Residues in bold represent those identified to be critical for the binding of NA by mAb CD6.

**Supplementary Table 4 Primers used for sequencing and mutagenesis of NA gene**

| Primers for NA gene cloning and sequencing | Sequence[a] |
|---|---|
| Ba-NA-1 | TATTCGTCTCAGGGAGCAAAAGCAGGAGT |
| 345R | CATGATATGAAAGGTTCTCTTATGACA |
| 830R | GCCAGTGTCTGGGTAACAGGAGCATTCCT |
| Ba-NA-1413R | ATATGGTCTCGTATTAGTAGAAACAAGGAGTTTTTT |
| 830F | AGGAATGCTCCTGTTACCCAGACACTGGC |
| Primers for site-directed mutagenesis | |
| P93S | GGCAATTCCTCTCTCTGCTCTGTTAGTGGATGGGCTATATAC |
| S95A | TCCTCTCTCTGCCCTGTTGCTGGATGGGCTATATACAG |
| S95N | TCCTCTCTCTGCCCTGTTAATGGATGGGCTATATACAG |
| R220K | GACACTATCAAGAGTTGGAAGAACAATATATTGAGACAC |
| N221K | CTATCAAGAGTTGGAGAAAGAATATATTGAGAACACAAGAG |
| Q250A | ACCGATGGACCAAGTAATGGAGCGGCCTCATACAAGATCTTC |
| I263V | CAGAATAGAAAAGGGAAAGGTAGTCAAATCAGTCGAAAG |
| I263K | CAGAATAGAAAAGGGAAAGAAAGTCAAATCAGTCGAAATG |
| V264T | GAATAGAAAAGGGAAAGATAACCAAATCAGTCGAAATGAATGCCCC |
| V267I | ATTAGGGGCATTCATTTCTATTGATTTGACTATCTTTCCC |
| N270D | GTCAAATCAGTCGAAATGGATGCCCCTAATTATCACTATGAG |
| W375A | AACGGTTTTGAGATGATTGCGGATCCGAACGGATGGACTGGG |
| W375G | C GGTTTTGAGATGATTGGGGATCCGAACGGATGGACTGGG |
| P377A | GAGATATTTGGGATGCGAACGGATGGACTGGGACAGAC |
| N378A | GAGATGATTTGGGATCCGGCCGGATGGACTGGGACAGAC |
| S388A | GGGACAGACAATAACTTCGCAATAAAGCAAGATATCGTAGG |
| I389V | GGGACAGACAATAACTTCTCAGTAAAGCAAGATATCGTAGG |
| N449D | ATATCCTTTTGTGGTGTAGACAGTGACACTGTGGGTTGGTC |
| N449E | GCATATCCTTTTGTGGTGTAGAGAGTGACACTGTGGGTTGG |
| N449Q | GCATATCCTTTTGTGGTGTACAGAGTGACACTGTGGGTTGG |
| N449K | GCATATCCTTTTGTGGTGTAAAAAGTGACACTGTGGGTTGGTC |
| D451G | TTTTGTGGTGTAGACAGTGGCACTGTGGGTTGGTCTTGGCC |
| N449D/D451G | TTTTGTGGTGTAGACAGTGGCACTGTGGGTTGGTCTTGGCC |

[a]Blue color highlights the nucleotide changes introduced to make the targeted amino acid mutations.

## References


1. Salerno, W.J., Seaver, S.M., Armstrong, B.R. & Radhakrishnan, I. MONSTER: inferring non-covalent interactions in macromolecular structures from atomic coordinate data. *Nucleic Acids Res* 32, W566-8 (2004).
2. Krissinel, E. & Henrick, K. Inference of macromolecular assemblies from crystalline state. *J Mol Biol* 372, 774-97 (2007).
3. McDonald, I.K. & Thornton, J.M. Satisfying hydrogen bonding potential in proteins. *J Mol Biol* 238, 777-93 (1994).