

We claim

1. A non-naturally occurring mutant neuraminidase (NA) polypeptide that improves expression and/or modifies the open/closed tetrameric conformational state of the NA polypeptide.
2. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
 - (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N1 NA polypeptide of SEQ ID NO:1, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, or all 7 of the following amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:
161T/A, 105A, 165T/I, 166P, 196Q, 203Y, 444V; or
 - (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N1 NA polypeptide of SEQ ID NO:1, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or all 17 of the following amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:
161T/A/V/S/T, 100L, 408M, 419V, 99P, 103N, 105A, 131Q/M, 163I/L, 165T/S/V/A/I, 166P, 177I, 196Q/T, 203Y, 205I, 442I, 444V.
3. The polypeptide of claim 2, wherein the polypeptide is
 - (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N1 NA polypeptide of SEQ ID NO:1, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, or all 7 of the following amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:
 - (i) **161T/A, 105A, 157K, 165T/I, 166P, 196Q, 203Y, 444V;** or
 - (ii) **105A, 165T/I, 166P, 196Q, 203Y, 444V.**
4. The polypeptide of claim 2, wherein the polypeptide is
 - (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N1 NA polypeptide of SEQ ID NO:1, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or all 17 of the following amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:

(i) **161T/A/V/S/T, 100L, 408M, 419V**, 99P, 103N, 105A, 131Q/M, 163I/L, 165T/S/V/A/I, 166P, 177I, 196Q/T, 203Y, 205I, 442I, 444V; or

(ii) 99P, 103N, 105A, 131Q/M, 163I/L, 165T/S/V/A/I, 166P, 177I, 196Q/T, 203Y, 205I, 442I, 444V.

5

5. The polypeptide of any one of claim 2-4, wherein the polypeptide includes one or more of the following sets of amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:

(i)

10 **161V/172A;**

100L/408M;

100L/408M/419V

103N/105A;

114I/166P;

15 101C/164C;

101C/164C/174V;

101C/122V/164C/174V/444V;

122V/444V;

131Q/442I;

20 101A/163L/444A;

131M/442I;

101A/131M/163L/442I/444A;

100L/101A/131M/163L/442I/444A

131M/163L/442I/444A;

25 100L/131M/163L/442I/444A;

99P/100L/161V/165S/172A/177I/196T/205I/408M/419V;

99P/100L/161V/165S/172A/177I/196T/408M/419V;

99P/100L/131Q/161V/165S/172A/177I/196T/205I/408M/419V;

99P/100L/131Q/161V/165S/172A/177I/196T/408M/419V;

30 99P/100L/101A/131M/161V/163L/165S/172A/177I/196T/205I/408M/419V/442I/444A;

99P/100L/101A/131M/161V/163L/165S/172A/177I/196T/408M/419V/442I/444A;

99P/161V/165S/172A/177I/196T/205I;

99P/161V/165S/172A/177I/196T;

99P/131Q/161V/165S/172A/177I/196T/205I;

- 99P/131Q/161V/165S/172A/177I/196T;
 99P/101A/131M/161V/163L/165S/172A/177I/196T/205I/442I/444A;
 99P/101A/131M/161V/163L/165S/172A/177I/196T/442I/444A;
 99P/196T;
- 5 99P/196T/205I;
 99P/177I/196T; and/or
 99P/177I/196T/205I; or
 (ii)
 103N/105A;
- 10 114I/166P;
 101C/164C;
 101C/164C/174V;
 101C/122V/164C/174V/444V;
 122V/444V;
- 15 131Q/442I;
 101A/1163L/444A;
 131M/442I;
 101A/131M/163L/442I/444A;
 100L/101A/131M/163L/442I/444A;
- 20 131M/163L/442I/444A;
 100L/131M/163L/442I/444A;
 99P/100L/161V/165S/172A/177I/196T/205I/408M/419V;
 99P/100L/131Q/161V/165S/172A/177I/196T/205I/408M/419V;
 99P/100L/131Q/161V/165S/172A/177I/196T/419V;
- 25 99P/100L/101A/131M/161V/163L/165S/172A/177I/196T/205I/408M/419V/442I/444A;
 99P/100L/101A/131M/161V/163L/165S/172A/177I/196T/408M/419V/442I/444A;
 99P/161V/165S/172A/177I/196T/205I;
 99P/161V/165S/172A/177I/196T;
 99P/131Q/161V/165S/172A/177I/196T/205I;
- 30 99P/131Q/161V/165S/172A/177I/196T;
 99P/101A/131M/161V/163L/165S/172A/177I/196T/205I/442I/444A;
 99P/101A/131M/161V/163L/165S/172A/177I/196T/442I/444A;
 99P/196T;
 99P/196T/205I;

99P/177I/196T; and/or
99P/177I/196T/205I.

6. The polypeptide of any one of claim 2-4, wherein the polypeptide includes three or
5 more of the listed amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1
or protocol 2.

7. The polypeptide of any one of claim 2-4, wherein the polypeptide includes five or
10 more of the listed amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1
or protocol 2.

8. The polypeptide of any one of claim 2-4, wherein the polypeptide includes seven or
15 more of the listed amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1
or protocol 2.

9. The polypeptide of any one of claim 2-4, wherein the polypeptide includes nine or
more of the listed amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1
or protocol 2.

10. The polypeptide of any one of claim 2-9, wherein the polypeptide further comprises 1,
2, 3, 4, 5, or all 6 of the following residues relative to SEQ ID NO:1 when aligned by
20 protocol 1 or protocol 2: 105S, 106V, 163I, 166V, 210G, 442S.

11. The polypeptide of any one of claim 2-10, wherein the polypeptide includes one or
25 more of the following sets of amino acid residues relative to SEQ ID NO:1 when aligned by
protocol 1 or protocol 2:

99P/100L/161V/165S/172A/177I/196T/205I/408M/419V;

99P/100L/161V/165S/172A/177I/196T/408M/419V;

99P/100L/131Q/161V/165S/172A/177I/196T/205I/408M/419V;

30 99P/100L/131Q/161V/165S/172A/177I/196T/408M/419V;

99P/100L/101A/131M/161V/163L/165S/172A/177I/196T/205I/408M/419V/442I/444A;

99P/100L/101A/131M/161V/163L/165S/172A/177I/196T/408M/419V/442I/444A;

99P/161V/165S/172A/177I/196T/205I;

- 99P/161V/165S/172A/177I/196T;
 99P/131Q/161V/165S/172A/177I/196T/205I;
 99P/131Q/161V/165S/172A/177I/196T/;
 99P/101A/131M/161V/163L/165S/172A/177I/196T/205I/442I/444A; and/or
 5 99P/101A/131M/161V/163L/165S/172A/177I/196T/442I/444A.

12. The non-naturally occurring polypeptide of any one of claims 2-11, wherein the polypeptide comprises the amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid selected from the group
 10 consisting of N1 mutants listed in Table 1 (SEQ ID NO:10-32 and 39-95), when aligned by protocol 1 or protocol 2, wherein the polypeptide includes all of the residues listed in Table 1 for an individual N1 mutant listed in Table 1.

13. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
 15 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N2 NA polypeptide of SEQ ID NO:2, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, or all 8 of the following amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2:
 101C, 131M, 162P, 165S/T, 166V, 195Q, 202Y, 443S; or

20 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N2 NA polypeptide of SEQ ID NO:2, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or all 12 of the following amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2:
 101C/A, 103N, 105A, 106V, 131M, 162P, 163I, 165S/T/A/I, 166V, 195Q, 202Y, 443S.

25

14. The polypeptide of claim 13, wherein the polypeptide is
 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N2 NA polypeptide of SEQ ID NO:2, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, or all 8 of the following amino acid residues
 30 relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2:
 101C, 131M, 162P, 165S/T, 166V, 195Q, 202Y, 443S.

15. The polypeptide of claim 13, wherein the polypeptide is

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N2 NA polypeptide of SEQ ID NO:2, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or all 12 of the following amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2:
- 5 101C/A, 103N, 105A, 106V, 131M, 162P, 163L, 165S/T/A/I, 166V, 195Q, 202Y, 443S.

16. The polypeptide of any one of claim 13-15, wherein the polypeptide includes one or more of the following sets of amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2:
- 10 103N/105A;
101C/164C;
101C/164C/173V;
101A/131M;
100L/101A/131M; and/or
- 15 100L/131M/163L.

17. The polypeptide of any one of claim 13-15, wherein the polypeptide includes three or more of the listed amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2.
- 20

18. The polypeptide of any one of claim 13-15, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2.

- 25 19. The polypeptide of any one of claim 13-15, wherein the polypeptide includes seven or more of the listed amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2.

20. The polypeptide of any one of claim 13-15, wherein the polypeptide includes nine or more of the listed amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2.
- 30

21. The non-naturally occurring polypeptide of any one of claims 13-20, wherein the polypeptide comprises the amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid selected from the group consisting of N2 mutants listed in Table 1 (SEQ ID NO:33-34), when aligned by protocol 1 or protocol 2, wherein the polypeptide includes all of the residues listed in Table 1 for an individual N2 mutant listed in Table 1.

5

22. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N2 NA polypeptide of SEQ ID NO:2, and wherein the non-naturally occurring polypeptide includes a 165Q/E amino acid residues relative to SEQ ID NO:2 when aligned by
10 protocol 1 or protocol 2, or includes 2 or 3 of 165Q/E, 176V, 195S amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2.

23. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
(a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
15 or 99% identical to the N3 NA polypeptide of SEQ ID NO:3, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2:
103N, 105S, 131Q/M, 157T, 165S/I, 166P, 196Q, 203Y, 205I, 443S, 445V; or
(b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
20 or 99% identical to the N3 NA polypeptide of SEQ ID NO:3, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:
103N, 105S, 106V, 131Q/M, 157T, 163L, 165S/I/V/A, 166P/V, 196Q, 203Y, 205I, 443S, 445V.

25

24. The polypeptide of claim 23, wherein the polypeptide is
(a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
or 99% identical to the N3 NA polypeptide of SEQ ID NO:3, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino
30 acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2:
103N, 105S, 131Q/M, 157T, 165S/I, 166P, 196Q, 203Y, 205I, 443S, 445V.

25. The polypeptide of claim 23, wherein the polypeptide is

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N3 NA polypeptide of SEQ ID NO:3, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2:
- 5 103N, 105S, 106V, 131Q/M, 157T, 163L, 165S/I/V/A, 166P/V, 196Q, 203Y, 205I, 443S, 445V.

26. The polypeptide of any one of claim 23-25, wherein the polypeptide includes one or more of the following sets of amino acid residues relative to SEQ ID NO:3 when aligned by
- 10 protocol 1 or protocol 2:
- 101C/164C;
101C/164C/174V;
101C/164C/174V/445V;
101A/445V;
- 15 101A/131M/445A;
131M/445A;
114I/166P;
101A/163L/445V;
101A/131M/163L/445A; and/or
- 20 131M/163L/445A.

27. The polypeptide of any one of claim 23-25, wherein the polypeptide includes three or more of the listed amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2.

25

28. The polypeptide of any one of claim 23-25, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2.

- 30 29. The polypeptide of any one of claim 23-25, wherein the polypeptide includes seven or more of the listed amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2.

30. The polypeptide of any one of claim 23-25, wherein the polypeptide includes nine or more of the listed amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2.
- 5 31. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N3 NA polypeptide of SEQ ID NO:3, and wherein the non-naturally occurring polypeptide includes 1, or both of the following amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2: 196S, 165Q/E.
- 10 32. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N4 NA polypeptide of SEQ ID NO:4, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the
- 15 following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2:
160V/S/T/A, 409M, 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P, 176I, 195Q, 202Y, 204I, 443I, 445V; or
- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N4 NA polypeptide of SEQ ID NO:4, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the
- 20 following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2:
160V/S/T/A, 409M, 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P/V, 176I, 195Q, 202Y, 204I, 443I, 445V.
- 25 33. The polypeptide of claim 32, wherein the polypeptide is
- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N4 NA polypeptide of SEQ ID NO:4, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the
- 30 following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2:
- (i) **160V/S/T/A, 409M**, 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P, 176I, 195Q, 202Y, 204I, 443I, 445V; or

(ii) 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P, 176I, 195Q, 202Y, 204I, 443I, 445V.

34. The polypeptide of claim 32, wherein the polypeptide is

5 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N4 NA polypeptide of SEQ ID NO:4, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2:

10 (i) **160V/S/T/A, 409M**, 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P/V, 176I, 195Q, 202Y, 204I, 443I, 445V; or

(ii) 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P/V, 176I, 195Q, 202Y, 204I, 443I, 445V.

15 35. The polypeptide of any one of claim 32-34, wherein the polypeptide includes one or more of the following sets of amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2:

(i)

160V/S/T/A;

20 **160V/171A;**

102N/104A;

100C/163C;

100C/163C/173V;

100C/163C/173V/445V;

25 130Q/443I;

100A/162L/445A;

130M/443I;

100A/130M/162L/443I/445A;

130M/162L/443I/445A;

30 176I/204I;

100C/121V/163C/173V/445V; and/or

121V/445V; or

(ii)

102N/104A;

- 100C/163C;
100C/163C/173V;
100C/163C/173V/445V;
130Q/443I;
5 100A/162L/445V;
130M/443I;
100A/130M/162L/443I/445A;
130M/162L/443I/445A;
176I/204I;
10 100C/121V/163C/173V/445V; and/or
121V/445V.

36. The polypeptide of any one of claim 32-34, wherein the polypeptide includes three or more of the listed amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1
15 or protocol 2.

37. The polypeptide of any one of claim 32-34, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1
20 or protocol 2.

38. The polypeptide of any one of claim 32-34, wherein the polypeptide includes seven or more of the listed amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1
or protocol 2.

39. The polypeptide of any one of claim 32-34, wherein the polypeptide includes nine or more of the listed amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1
25 or protocol 2.

40. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at
30 least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N4 NA polypeptide of SEQ ID NO:4, and wherein the non-naturally occurring polypeptide includes 1, or both of the following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2: 164Q/E, 195S.

41. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally
 occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following
 5 amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:
97L, 410M, 96P, 98A, 100N, 102S/A, 110D, 128Q/M, 160I, 162V/A/I, 163V/P, 193Q/T,
 445I; or

(b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally
 10 occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the
 following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or
 protocol 2:
97L, 410M, 96P, 98A, 100N, 102S/A, 103V, 110D, 128Q/M, 154T, 160I, 162V/A/I/T,
 163V/P, 174I, 193Q/T, 445I.

15

42. The polypeptide of claim 41, wherein the polypeptide is
 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally
 occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following
 20 amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:

(i) **97L, 410M**, 96P, 98A, 100N, 102S/A, 110D, 128Q/M, 160I, 162V/A/I, 163V/P,
 193Q/T, 445I; or

(ii) 96P, 98A, 100N, 102S/A, 110D, 128Q/M, 160I, 162V/A/I, 163V/P, 193Q/T,
 445I.

25

43. The polypeptide of claim 41, wherein the polypeptide is
 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally
 occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the
 30 following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or
 protocol 2:

(i) **97L, 410M**, 96P, 98A, 100N, 102S/A, 103V, 110D, 128Q/M, 154T, 160I,
 162V/A/I/T, 163V/P, 174I, 193Q/T, 445I; or

(ii) 96P, 98A, 100N, 102S/A, 103V, 110D, 128Q/M, 154T, 160I, 162V/A/I/T, 163V/P, 174I, 193Q/T, 445I.

44. The polypeptide of any one of claim 41-43, wherein the polypeptide includes one or
5 more of the following sets of amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:

(i)

97L/410M;
100N/102A;
10 98C/161C;
128Q/445I;
128M/445I;
98A/128M/445I/447A;
97L/98A/128M/445I/447A;
15 128M/445I/447A;
97L/128M/445I/447A;
96P/193T;
111I/163P;
96P/193T/202I;
20 96P/174I/193T; and/or
96P/174I/193T/202I; or

(ii)

100N/102A;
98C/161C;
25 128Q/445I;
128M/445I;
98A/128M/445I/447A;
97L/98A/128M/445I/447A;
128M/445I/447A;
30 97L/128M/445I/447A;
96P/193T;
111I/163P;
96P/193T/202I;
96P/174I/193T; and/or

96P/174I/193T/202L

45. The polypeptide of any one of claim 41-43, wherein the polypeptide includes three or more of the listed amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2.
46. The polypeptide of any one of claim 41-43, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2.
47. The polypeptide of any one of claim 41-43, wherein the polypeptide includes seven or more of the listed amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2.
48. The polypeptide of any one of claim 41-43, wherein the polypeptide includes nine or more of the listed amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2.
49. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 1, or both of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2: 162 Q/E, 200S.
50. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2: 99P, 103N, 113D, 131Q, 161I, 162P, 163I, 165S/T/V/A, 196Q, 203Y, 445S; or
 - (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the

following amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:

99P, 103N, 105S, 106V, 113D, 131Q, 157T, 161I, 162P, 163I/L, 165S/T/V/A/I, 166V/P, 196Q, 203Y, 445S.

5

51. The polypeptide of claim 50, wherein the polypeptide is

(a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino

10 acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:

99P, 103N, 113D, 131Q, 161I, 162P, 163I, 165S/T/V/A, 196Q, 203Y, 445S.

52. The polypeptide of claim 50, wherein the polypeptide is

(b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all 15 of the

15 following amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:

99P, 103N, 105S, 106V, 113D, 131Q, 157T, 161I, 162P, 163I/L, 165S/T/V/A/I,

20 166V/P, 196Q, 203Y, 445S.

53. The polypeptide of any one of claim 50-52, wherein the polypeptide includes one or more of the following sets of amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:

25 101C/164C;

101C/164C/174V;

101C/164C/174V/447V;

122V/447V;

101A/163L;

30 99P/196T; and/or

99P/196T/205I.

54. The polypeptide of any one of claim 50-52, wherein the polypeptide includes three or more of the listed amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2.
- 5 55. The polypeptide of any one of claim 50-52, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2.
56. The polypeptide of any one of claim 50-52, wherein the polypeptide includes seven or
10 more of the listed amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2.
57. The polypeptide of any one of claim 50-52, wherein the polypeptide includes nine or more of the listed amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1
15 or protocol 2.
58. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring
20 polypeptide includes a 165E amino acid mutation relative to SEQ ID NO:6 when aligned by protocol 1, and optionally also includes a 177V amino acid mutation relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2.
59. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
25 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:
98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 202Y, 448V; or
30 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:
98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 176I, 202Y, 446I, 448V.

60. The polypeptide of claim 59, wherein the polypeptide is
 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally
 occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino
 5 acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:
 98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 202Y, 448V.

61. The polypeptide of claim 59, wherein the polypeptide is
 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 10 or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally
 occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following
 amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:
 98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 176I, 202Y, 446I, 448V.

15 62. The polypeptide of any one of claim 59-61, wherein the polypeptide includes one or
 more of the following sets of amino acid residues relative to SEQ ID NO:7 when aligned by
 protocol 1 or protocol 2:
 100C/163C;
 100C/163C/173V;
 20 100C/163C/173V/448V;
 99L/446I/448A;
 98P/195T;
 130Q/446I;
 446I/448A;
 25 98P/195T/204I;
 98P/176I/195T; and/or
 98P/176I/195T/204I.

30 63. The polypeptide of any one of claim 59-61, wherein the polypeptide includes three or
 more of the listed amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1
 or protocol 2.

64. The polypeptide of any one of claim 59-61, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2.
- 5 65. The polypeptide of any one of claim 59-61, wherein the polypeptide includes seven or more of the listed amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2.
66. The polypeptide of any one of claim 59-61, wherein the polypeptide includes nine or
10 more of the listed amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2.
67. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical
15 to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally occurring polypeptide includes one or both of the following amino acid mutation relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2: 164Q/E, 195S.
68. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
20 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:
408M, 101N, 103A/S, 111D, 129Q, 161P/L, 163S/T/V/A/I, 164V, 194Q, 201Y, 442I; or
25 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all 15 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:
30 **98L**, **408M**, 101N, 103A/S, 104V, 111D, 129Q/M, 161P/L, 163S/T/V/A/I/S/T, 164V/P, 175I, 194Q, 201Y, 203I, 442I

69. The polypeptide of claim 68, wherein the polypeptide is

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

(i) **408M**, 101N, 103A/S, 111D, 129Q, 160P, 161L, 163S/T/V/A/I, 164V, 194Q, 201Y, 442I; or

(ii) 101N, 103A/S, 111D, 129Q, 160P, 161L, 163S/T/V/A/I, 164V, 194Q, 201Y, 442I.

10

70. The polypeptide of claim 68, wherein the polypeptide is

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all 15 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

(i) **98L, 408M**, 101N, 103A/S, 104V, 111D, 129Q/M, 160P, 161L, 163S/T/V/A/I/S/T, 164V/P, 175I, 194Q, 201Y, 203I, 442I; or

(ii) 101N, 103A/S, 104V, 111D, 129Q/M, 160P, 161L, 163S/T/V/A/I/S/T, 164V/P, 175I, 194Q, 201Y, 203I, 442I

20

71. The polypeptide of any one of claim 68-70, wherein the polypeptide includes one or more of the following sets of amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

25 (i)

98L/408M;

160P/163S;

101N/103A;

99C/162C;

30 99C/162C/172V;

129Q/442I;

99A/161L;

99A/161L/442I;

161L/442I;

- 129M/442I;
 99A/129M/161L/442I/444A;
 98L/99A/129M/161L/442I/444A;
 129M/161L/442I/444A;
 5 98L/129M/161L/442I/444A; and/or
 175I/203I; or
 (ii)
 160P/163S;
 101N/103A;
 10 99C/162C;
 99C/162C/172V;
 129Q/442I;
 99A/161L;
 99A/161L/442I;
 15 161L/442I;
 129M/442I
 99A/129M/161L/442I/444A;
 98L/99A/129M/161L/442I/444A;
 129M/161L/442I/444A;
 20 98L/129M/161L/442I/444A; and/or
 175I/203I.

72. The polypeptide of any one of claim 68-70, wherein the polypeptide includes three or more of the listed amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1
 25 or protocol 2.

73. The polypeptide of any one of claim 68-70, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1
 30 or protocol 2.

74. The polypeptide of any one of claim 68-70, wherein the polypeptide includes seven or more of the listed amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1
 or protocol 2.

75. The polypeptide of any one of claim 68-70, wherein the polypeptide includes nine or more of the listed amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2.
- 5 76. The non-naturally occurring polypeptide of any one of claims 80-85, wherein the polypeptide comprises the amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid selected from the group consisting of N8 mutants listed in Table 1 (SEQ ID NO:35-38), when aligned by protocol 1, wherein the polypeptide includes all of the residues listed in Table 1 for an individual N8
10 mutant listed in Table 1.
77. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring
15 polypeptide includes a 163E amino acid mutation relative to SEQ ID NO:8 when aligned by protocol 1, and further may optionally include a 194S mutation relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2.
78. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
20 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, or all 10 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:
94P, 95L, 100S, 126Q/M, 160V/A/I, 161V, 191Q, 198Y, 439S, 441V; or
25 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all 14 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:
94P, 95L, 98N, 100S/A, 126Q/M, 152T, 158I, 160V/A/I/T, 161V, 191Q, 198Y, 200I, 439S,
30 441V.
79. The polypeptide of claim 78, wherein the polypeptide is
(a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally

occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, or all 10 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:

94P, 95L, 100S, 126Q/M, 160V/A/I, 161V, 191Q, 198Y, 439S, 441V.

5 80. The polypeptide of claim 78, wherein the polypeptide is

(b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all 14 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:

10 94P, 95L, 98N, 100S/A, 126Q/M, 152T, 158I, 160V/A/I/T, 161V, 191Q, 198Y, 200I, 439S, 441V.

81. The polypeptide of any one of claim 78-80, wherein the polypeptide includes one or more of the following sets of amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:

15 96C/159C;

96C/159C/441V;

96A/441A;

96A/126M/439I/441A;

20 95L/96A/126M/439I/441A;

126M/439I/441A;

95L/126M/439I/441A;

94P/191T;

98N/100A; and/or

25 94P/191T/200I.

82. The polypeptide of any one of claim 78-80, wherein the polypeptide includes three or more of the listed amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2.

30

83. The polypeptide of any one of claim 78-80, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2.

84. The polypeptide of any one of claim 78-80, wherein the polypeptide includes seven or more of the listed amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2.
- 5 85. The polypeptide of any one of claim 78-80, wherein the polypeptide includes nine or more of the listed amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2.
86. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at
10 least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes a 160Q amino acid mutation relative to SEQ ID NO:9 when aligned by protocol 1, or may include a combination of 160Q/E and 172V amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2.
- 15 87. A composition, comprising one or more of the polypeptides of any preceding claim linked to a scaffold.
88. The composition of claim 87, wherein the scaffold comprises a protein scaffold.
20
89. The composition of claim 88, wherein the polypeptide is covalently linked to a protein subunit of the protein scaffold to form a fusion protein.
90. A nucleic acid encoding the polypeptide of any preceding claim, or the fusion protein
25 of claim 89.
91. An expression vector comprising the nucleic acid of claim 90 operatively linked to a suitable control sequence.
- 30 92. A host cell comprising the nucleic acid of claim 90, the expression vector of claim 91, and/or the polypeptide of any preceding claim.
93. A pharmaceutical composition, comprising

(a) one or more of the polypeptides, fusion protein, composition, nucleic acid, expression vector, and/or the host cell of any preceding claim; and

(b) a pharmaceutically acceptable carrier.

5 94. A vaccine comprising

(a) one or more of the polypeptides, fusion protein, composition, nucleic acid, expression vector, and/or the host cell of any preceding claim; and

(b) a pharmaceutically acceptable carrier.

10 95. A method for treating or limiting development of an influenza infection, comprising administering to a subject in need thereof an amount effective to treat or limit development of the influenza infection of a polypeptide, fusion protein, composition, vaccine, nucleic acid, expression vector, host cell, pharmaceutical composition, and/or vaccine of any preceding claim.

15