

a PA/P3 subunit

A/WSN/1933 (H1N1) 1 M E . D F V . . . R Q C F N P M I V E L A E K A M K E Y G E D L K I E T N K F A A I C T H L E V C F M Y S D F H F I D E
C/Johannesburg/1/1966 1 M S K T F A E I A E A F L E E A V R I A K E A V E Y G D H E R K I I Q I G I H F Q V C C M F C D . E Y L S T

A/WSN/1933 (H1N1) 57 Q G E S I V V E L G D P N A L L K H R F E I E G R D R T I A W T V I N S I C N T T G A E K P K F L P D L Y D Y K K N R
C/Johannesburg/1/1966 56 N G S D R F V L I E G R K R G T A V S L Q N E L C K S Y D L E P L P F L C D I F D R E E K Q

A/WSN/1933 (H1N1) 117 F I E I G V T R R E V H I Y Y L E K A N K I K S E K T H I H I F S F T G E E M A T K A D Y T L D . . . E E S R A R I K T
C/Johannesburg/1/1966 102 F V E I G I T R K A D D S Y F Q S K F G K L . G N S C K I F V F S Y D G R L D K N C E G P M E E Q K L R I F S

A/WSN/1933 (H1N1) 174 R I F T I R Q E M A S R G L W D S F R Q S E R G E E T I E E R F E I T G T M R K L A D Q S L E P N F S S L E N F R A Y V
C/Johannesburg/1/1966 156 F L A T A A D F L R K E N M F N E I F L P D N E E T I E . . M K K G K F F L E L R D E S V E L P F Q T Y E Q M K D Y C

A/WSN/1933 (H1N1) 234 D G F E P N G Y . I E G K L S O M S K E V N A R I E P F L K S T P R P L R L P D G P P C S Q R S K F L L M D A L K L S .
C/Johannesburg/1/1966 214 E K F K G N P R E L A S K V S Q M Q S N I K L P I K H Y E Q N K F R Q I R L P K G P M A P Y T H K F L M E B A W M F T K

A/WSN/1933 (H1N1) 292 I E D P S H E G E G I P L Y D A I K C M R T F F G W K E P N V V P P E K G I N P N Y . L L . . S . W K Q V L A E L Q D
C/Johannesburg/1/1966 274 I S D P E R S R A G E I L D F F K K G N L S A I R P K D K P L Q G K Y P I H Y K N L W N O I K A . . . A

A/WSN/1933 (H1N1) 348 I E N E E K I P R T K N M K K T S Q L K W A L G E N M A P E K V D F D D C K D V G D L K Q Y D S D E P E I R S L
C/Johannesburg/1/1966 324 I A D R T M V I N E N D H S E F L G G I G R A S K K I P E I S L T Q D V I T T E G L K Q S E N K L E P P R S F

A/WSN/1933 (H1N1) 404 A S W I Q N E . . F . N K A C E L T D S S W I E L D E I G E D A A P I E H I A S M R R N Y F T A E V S H C R A T E Y I M
C/Johannesburg/1/1966 379 P R W F N A E W M W A I K D S D L T . . G W V P M A E Y P P A D N E L E D Y A E H L N K T M E G V L Q G T N C A R E M G

A/WSN/1933 (H1N1) 461 K G V Y I N T A L L N A S C A A M D D F Q L I P M I S K C R T K E G R R K T N I Y G F I I K G R S H L R
C/Johannesburg/1/1966 437 K C I L T V G A L M T E C R L F P G K I K V V P I Y A R S K E R K S M Q E G L P V P S E M D C I F G I C V K S K S H L N

A/WSN/1933 (H1N1) 513 N D T D V V N F V S M E F S L T P R L E P H K W E K Y C V L E V G D M L R S A I G H V S R P M F L Y V R T N
C/Johannesburg/1/1966 497 K D D G M Y T I I T F E F S I R P N L E . . K H Q K Y T V F E A G H T T V R M K K G E S V I G R E V P L Y L Y C R T T

A/WSN/1933 (H1N1) 569 G T S K I K M K W G M E M R R C L L Q S L Q Q I E S M I E A E S S V K E K D M T K E F F E N K S E T W P V G E S P K G V
C/Johannesburg/1/1966 555 A L S K I K N D W L S K A R R C F I T T M D T V E T I C L R E S S A K A E E N L V E K T L N . . E K Q M W I C K K N G E L

A/WSN/1933 (H1N1) 629 E E G S I G K V C R T L L A K S V F N S L Y A S P Q L E G F S A E S R K L L L I V Q A L R D N L E P G T F D L G G L Y E
C/Johannesburg/1/1966 613 I A Q P L R E A L R V Q L V Q Q F Y F C I Y N D S Q L E G F C N E Q K K I L M A L E G D K R N K S S F G F N P E G L L E

A/WSN/1933 (H1N1) 689 A I E E C L I N D P W V L L N A S W F N S F L T H A L R
C/Johannesburg/1/1966 673 K I E E C L I N P M C L F M A Q R L N E L V I E A S K R G A K F F K T D

b PB1 subunit

A/WSN/1933 (H1N1) 1 MDVNP[•]T[•]L[•]L[•]F[•]L[•]KVPAQNA[•]ISTT[•]F[•]P[•]Y[•]T[•]G[•]D[•]P[•]P[•]Y[•]SHGT[•]G[•]T[•]G[•]Y[•]T[•]M[•]D[•]T[•]V[•]N[•]R[•]T[•]H[•]Q[•]Y[•]SERGRWT[•]T[•]N[•]T[•]E[•]
C/Johannesburg/1/1966 1 MEINP[•]Y[•]L[•]M[•]F[•]L[•]NNDV[•]T[•]SL[•]ISTT[•]F[•]P[•]Y[•]T[•]G[•]PP[•]MSHG[•]SS[•]T[•]K[•]Y[•]T[•]LE[•]T[•]I[•]K[•]R[•]T[•]Y[•]D[•]YS[•]RTSVEK[•]T[•]SKV[•]

A/WSN/1933 (H1N1) 61 TGAP[•]Q[•]LNP[•]IDG[•]. . P[•]L[•]F[•]ED[•]NE[•]PS[•]GYA[•]Q[•]TDC[•]V[•]TE[•]AMAF[•]LE[•]. SHP[•]GI[•]F[•]ETS[•]CL[•]ET[•]MEV[•]V[•]Q[•]QT[•]
C/Johannesburg/1/1966 61 FNIP[•]RRK[•]FC[•]NC[•]LED[•]KD[•]EL[•]V[•]K[•]PT[•]GN[•]V[•]DISS[•]LE[•]GLA[•]EM[•]ME[•]K[•]RM[•]G[•]E[•]GF[•]PK[•]H[•]CV[•]ME[•]AE[•]TE[•]IL[•]KM[•]

A/WSN/1933 (H1N1) 118 RVDK[•]L[•]T[•]Q[•]GR[•]Q[•]T[•]Y[•]D[•]WT[•]LN[•]R[•]N[•]Q[•]PA[•]ATA[•]L[•]AN[•]T[•]IE[•]V[•]FR[•]S[•]N[•]GL[•]TAN[•]ES[•]GR[•]L[•]ID[•]F[•]L[•]K[•]D[•]V[•]M[•]ES[•]M[•]N[•]KE[•]
C/Johannesburg/1/1966 121 HFS[•]R[•]L[•]T[•]EG[•]R[•]Q[•]T[•]Y[•]D[•]WT[•]SE[•]R[•]N[•]M[•]PA[•]ATA[•]L[•]Q[•]L[•]T[•]V[•]DA[•]I[•]K[•]ET[•]E[•]GP[•]. K[•]GT[•]T[•]M[•]L[•]E[•]Y[•]C[•]N[•]K[•]M[•]I[•]E[•]M[•]L[•]D[•]W[•]K

A/WSN/1933 (H1N1) 178 EME[•]I[•]T[•]H[•]F[•]Q[•]R[•]K[•]RR[•]V[•]R[•]D[•]N[•]M[•]T[•]K[•]M[•]V[•]T[•]Q[•]R[•]T[•]I[•]G[•]K[•]R[•]K[•]Q[•]R[•]L[•]N[•]K[•]R[•]S[•]Y[•]L[•]I[•]R[•]A[•]L[•]T[•]L[•]N[•]T[•]M[•]T[•]K[•]D[•]A[•]E[•]R[•]G[•]K[•]L[•]K[•]
C/Johannesburg/1/1966 180 EIK[•]FK[•]K[•]V[•]K[•]T[•]V[•]V[•]RR[•]E[•]K[•]D[•]K[•]R[•]S[•]G[•]KE[•]L[•]K[•]T[•]K[•]V[•]P[•]V[•]M[•]G[•]I[•]D[•]S[•]IK[•]H[•]D[•]E[•]F[•]L[•]I[•]R[•]A[•]L[•]T[•]L[•]N[•]T[•]M[•]A[•]K[•]D[•]G[•]E[•]R[•]G[•]K[•]L[•]Q

A/WSN/1933 (H1N1) 238 RRAI[•]AT[•]PG[•]M[•]Q[•]IR[•]GF[•]V[•]Y[•]F[•]V[•]ET[•]LA[•]RS[•]ICE[•]KL[•]E[•]Q[•]SGL[•]P[•]V[•]GG[•]NE[•]KK[•]AK[•]L[•]AN[•]V[•]R[•]K[•]M[•]M[•]T[•]NS[•]Q[•]D[•]TE[•]
C/Johannesburg/1/1966 240 RRAI[•]AT[•]PG[•]M[•]IV[•]RP[•]FS[•]K[•]IV[•]ET[•]VA[•]Q[•]K[•]ICE[•]KL[•]KE[•]SGL[•]P[•]V[•]GG[•]NE[•]KK[•]AK[•]L[•]KT[•]TV[•]TS[•]LN[•]ARM[•]NS[•]D[•]Q

A/WSN/1933 (H1N1) 298 ISFT[•]IT[•]GD[•]NT[•]K[•]W[•]NE[•]N[•]Q[•]N[•]P[•]RM[•]F[•]L[•]AM[•]ITY[•]Y[•]IT[•]R[•]N[•]Q[•]PE[•]W[•]FR[•]N[•]V[•]L[•]SI[•]AP[•]IM[•]ES[•]N[•]K[•]M[•]AR[•]L[•]G[•]K[•]G[•]Y[•]MF[•]
C/Johannesburg/1/1966 300 FAV[•]NI[•]T[•]GD[•]NS[•]SK[•]W[•]NE[•]C[•]Q[•]PE[•]AV[•]LA[•]L[•]L[•]AY[•]IT[•]K[•]DS[•]SD[•]LM[•]K[•]D[•]LC[•]SV[•]AP[•]VE[•]ES[•]C[•]N[•]K[•]F[•]V[•]RL[•]GG[•]Q[•]IR[•]L

A/WSN/1933 (H1N1) 358 ESK[•]SM[•]K[•]L[•]R[•]T[•]Q[•]IP[•]AE[•]ML[•]AS[•]ID[•]L[•]K[•]Y[•]FN[•]D[•]ST[•]R[•]KK[•]IE[•]E[•]K[•]IR[•]P[•]L[•]L[•]ID[•]GT[•]AS[•]LS[•]EG[•]MM[•]MG[•]MF[•]N[•]M[•]LS[•]T[•]
C/Johannesburg/1/1966 360 SN[•]K[•]R[•]KT[•]KE[•]VI[•]IK[•]AE[•]K[•]M[•]G[•]K[•]Y[•]. N[•]LM[•]RE[•]E[•]Y[•]K[•]N[•]LF[•]E[•]PLE[•]K[•]Y[•]I[•]Q[•]K[•]D[•]V[•]CF[•]LP[•]GG[•]ML[•]MG[•]MF[•]N[•]M[•]LS[•]T[•]

A/WSN/1933 (H1N1) 418 VLG[•]VS[•]I[•]TL[•]N[•]L[•]G[•]Q[•]R[•]H[•]T[•]K[•]T[•]TY[•]W[•]WD[•]GL[•]Q[•]SS[•]DD[•]F[•]AL[•]IV[•]NA[•]P[•]N[•]HE[•]G[•]I[•]Q[•]AG[•]V[•]N[•]RF[•]Y[•]RT[•]CK[•]L[•]L[•]GIN[•]M[•]
C/Johannesburg/1/1966 419 VLG[•]VS[•]T[•]LC[•]Y[•]M[•]DE[•]EL[•]K[•]AK[•]G[•]CF[•]WT[•]GL[•]Q[•]SS[•]DD[•]F[•]VL[•]FA[•]VA[•]SN[•]WS[•]NI[•]H[•]WT[•]IR[•]RF[•]NAV[•]CK[•]L[•]L[•]GIN[•]M[•]

A/WSN/1933 (H1N1) 478 SKK[•]K[•]S[•]Y[•]I[•]N[•]R[•]T[•]GT[•]FE[•]FT[•]S[•]FF[•]Y[•]R[•]Y[•]G[•]F[•]V[•]AN[•]FS[•]M[•]EL[•]P[•]S[•]F[•]GV[•]S[•]C[•]I[•]NE[•]S[•]AD[•]MS[•]I[•]G[•]V[•]T[•]V[•]I[•]K[•]N[•]M[•]IN[•]N[•]
C/Johannesburg/1/1966 479 SLE[•]K[•]S[•]Y[•]G[•]S[•]L[•]PE[•]L[•]FE[•]FT[•]S[•]MF[•]F[•]D[•]GE[•]F[•]V[•]SN[•]LA[•]M[•]EL[•]P[•]AF[•]TT[•]AG[•]V[•]NE[•]GV[•]DF[•]TA[•]AM[•]SI[•]IK[•]T[•]N[•]M[•]IN[•]N[•]

A/WSN/1933 (H1N1) 538 DLG[•]PA[•]T[•]A[•]Q[•]M[•]AL[•]Q[•]L[•]F[•]IK[•]D[•]Y[•]R[•]Y[•]TY[•]R[•]GH[•]R[•]G[•]D[•]T[•]Q[•]I[•]Q[•]T[•]RR[•]S[•]FE[•]TK[•]KL[•]WE[•]Q[•]TH[•]SK[•]AG[•]LL[•]V[•]SD[•]GG[•]PN[•]
C/Johannesburg/1/1966 539 SLS[•]PS[•]T[•]AL[•]M[•]AL[•]RI[•]CL[•]Q[•]E[•]FR[•]AT[•]Y[•]R[•]VP[•]W[•]DS[•]RV[•]K[•]GG[•]RM[•]K[•]IN[•]EF[•]IK[•]T[•]IE[•]N[•]K[•]D[•]GL[•]L[•]L[•]AD[•]GG[•]KL

A/WSN/1933 (H1N1) 598 LY[•]NI[•]R[•]N[•]L[•]HI[•]PE[•]V[•]CL[•]KE[•]EL[•]M[•]DE[•]Y[•]Q[•]GR[•]LC[•]NP[•]L[•]NP[•]FF[•]V[•]N[•]H[•]K[•]D[•]IES[•]V[•]NN[•]AV[•]IMP[•]A[•]H[•]GP[•]AK[•]N[•]ME[•]Y[•]
C/Johannesburg/1/1966 599 MN[•]NI[•]ST[•]L[•]HI[•]PE[•]EV[•]L[•]KE[•]E[•]K[•]M[•]DE[•]Q[•]Y[•]R[•]NR[•]RV[•]FN[•]PN[•]PF[•]T[•]N[•]FD[•]KT[•]. . . . ID[•]TF[•]RA[•]H[•]GP[•]IR[•]VE[•]EN

A/WSN/1933 (H1N1) 658 DAV[•]AT[•]TH[•]SW[•]IP[•]K[•]R[•]NR[•]S[•]I[•]L[•]NT[•]SO[•]R[•]G[•]I[•]LE[•]EQ[•]Y[•]Q[•]K[•]C[•]NI[•]E[•]E[•]K[•]FF[•]PS[•]SS[•]Y[•]RR[•]P[•]V[•]GI[•]SS[•]M[•]VE[•]EA[•]
C/Johannesburg/1/1966 654 EAV[•]VS[•]TH[•]S[•]FR[•]TR[•]AN[•]RT[•]L[•]NT[•]DM[•]RAM[•]MA[•]E[•]E[•]K[•]RY[•]Q[•]M[•]V[•]CD[•]ME[•]K[•]SV[•]FE[•]S[•]AD[•]IN[•]PP[•]IG[•]AM[•]SI[•]GE[•]A

A/WSN/1933 (H1N1) 718 MVS[•]R[•]AR[•]ID[•]AR[•]ID[•]F[•]ES[•]GR[•]IK[•]KE[•]E[•]FT[•]E[•]IM[•]K[•]IC[•]ST[•]IE[•]EL[•]RR[•]Q[•]K[•].
C/Johannesburg/1/1966 714 IEE[•]K[•]LLER[•]AK[•]M[•]K[•]R[•]D[•]IG[•]AL[•]IED[•]SE[•]YE[•]E[•]IK[•]D[•]I[•]IRD[•]AK[•]K[•]AR[•]LES[•]R

c PB2 subunit

A/WSN/1933 (H1N1)	1 M E R I K E L R N L M S Q S R T R E I L T K T T V D H M A I I K K Y T S G R Q E K N P A L R M K W M M A M K Y P
C/Johannesburg/1/1966	1	M S L L L T I A K E Y K R L C Q D A K A A Q M M T V G T V S N Y T T F K K W T S R K E K N P S L R M R W A M S S K F P
A/WSN/1933 (H1N1)	57	T T A D K R I T E M I P E . R N E Q G Q T L W S K M N D A G . S D R V M V S P L A V T W W N R N G P V T S T V . H Y P K
C/Johannesburg/1/1966	61	T I A N K R M L E E A Q I P K E H N N V A L W E D T E D V S K R D H V L A S A S C I N Y W N F C G P C V N N S E V I K E
A/WSN/1933 (H1N1)	114	I Y K T Y F E K V E R L K H G T F G P V H F R N Q V K I R R R V D I N P G H A D L S A K E A Q D V I M E V V F P N E V G
C/Johannesburg/1/1966	121	V Y K S R F G R L E R R K E I M W K E L R F T L V D R Q R R R V D T Q P V E Q R L R T G E I K D L Q M W T L E D E A P
A/WSN/1933 (H1N1)	174	A R I L T S E S Q L T T T K E K K E E L Q G C K I S P L M V A Y M L I E R E L V R K T R F L P V A G G T S S V Y I E V L H
C/Johannesburg/1/1966	181	L A S K F I L D N Y G L V K E M R S K F A N K P L N K E V V A H M L E K Q F N P E S R F L P V F G A I R P E R M E L I H
A/WSN/1933 (H1N1)	234	L T Q G T C W E Q M Y T P G C E A R N D D V D Q S L I I A A R N I V R R A T V S A . D P L A S T L E M C H S T . . Q I G
C/Johannesburg/1/1966	241	A L G E T W T Q E A N T A G I S N V D Q R K N D I R A V C R K V C L A A N A S I M N A K S K L V E Y I K S T S M R I G
A/WSN/1933 (H1N1)	291	G . . I R M V N I L R Q N P T E E Q A V D I C K A M G L R I S S S F S F G G T F K R T S G S S V K R E E E V L T G N
C/Johannesburg/1/1966	301	E T E R K L E E L I L E T D D V S P E V T I C K S A L G G Q L G K T L S F G P M L L K K I S G S G V K V K D T V Y I Q G
A/WSN/1933 (H1N1)	349	L Q T L K I R V H E G Y E E F T M V G R R A T A I L R K A T R R L I Q L I V S G R D E Q S I A E A I I V A M V F S Q E D
C/Johannesburg/1/1966	361	V R A V Q F E Y W S E Q E E F Y G E Y K S A T A L F S R K B R S L E W I T I G G I N E D R K R L L A M C M F C R D G
A/WSN/1933 (H1N1)	409	C M I K A V R G D L N F V N R A N Q R L N P M H Q L L R H F Q K . . D A K V L F Q N W G I E S I D N V M G M I . . . G I
C/Johannesburg/1/1966	421	D Y F K D A P A T I T M A D L S T K L G R E I P Y Q Y V M M N W I Q K S E D N L E A L L Y S R G I
A/WSN/1933 (H1N1)	464	L . . . P D M T P S T E M S M R G V R I S K M G V D E Y S S A E K I V V S I D R F L R V R D Q R G N V
C/Johannesburg/1/1966	470	V E T N P G K M G S S M G I D G S K R A I K S I L R A V T I Q S G K I D M P E S K E K I H L E L S D N L E A F D S S G R I
A/WSN/1933 (H1N1)	512	L L S P E E V S E T Q G T E K L T I T Y S S S M M W E I N G P E S V L V N T Y Q W I I R N W E T V K I Q W S Q
C/Johannesburg/1/1966	530	V A T I L D L P S D K K V T F Q D V S F Q H P D L A V L R D E K T A I T K G Y E A L I K R L G T G D N D I P S L I A K K
A/WSN/1933 (H1N1)	567	N P T M L Y N K M E F E P F Q S I V P K A V R G C Y S G F V R T I F Q Q M R D V L G T F D T A Q I I K L L P F A A A P P
C/Johannesburg/1/1966	590	D Y L S L Y N L P E V K L M A P I I R P N R R G V Y S R V A R K L V S . T Q V T T G H Y S L H E L I K V L P F T Y F A P
A/WSN/1933 (H1N1)	627	K Q S G M Q F S S L T I N V R G S G M R I L V R G N S P V F N Y N K T T K R L T . V L G K D . A G P L T E D P D E G T A
C/Johannesburg/1/1966	649	K Q G M F E G R L F F S N D F V E P G V N N N V F S W S K A D S S K I Y C H G I A I R V P L V V . . G D E H M
A/WSN/1933 (H1N1)	685	G V E S A V L R G F L I L G K E D R R Y G P A L S I N E L S N L A K G E K A N V L I G Q G D V V L V M K R K R N S S I L
C/Johannesburg/1/1966	703	D T S L A L L E G F S V C E N D . . P R A P M V T R Q D L I D V G F G Q K V R L F V G Q G S V R T F K R T A S Q R A S
A/WSN/1933 (H1N1)	745	T D S Q T A T K R I R M A I N
C/Johannesburg/1/1966	761	S D V N K N V K K I K M S N .

Supplementary Fig. 1 Sequence alignment of FluPol_A and FluPol_C. a - c, Sequence alignment of FluPol subunits (a, PA/P3; b, PB1; c, PB2) from influenza A/WSN/33 (H1N1) and C/Johannesburg/1/1966 viruses. Residues involved in forming the asymmetric FluPol dimer interface are highlighted in cyan, and residues involved in ANP32A binding are highlighted in orange. The figure was prepared with Espright 3.0⁶⁰.