

Supplementary Figure 1.

Alignment showing putative PA-X protein sequences between H1N1-NC, H1N2-NC, and H3N2-NC viruses. The frameshift (-X) encoded region is boxed. Amino acids are color-coded.

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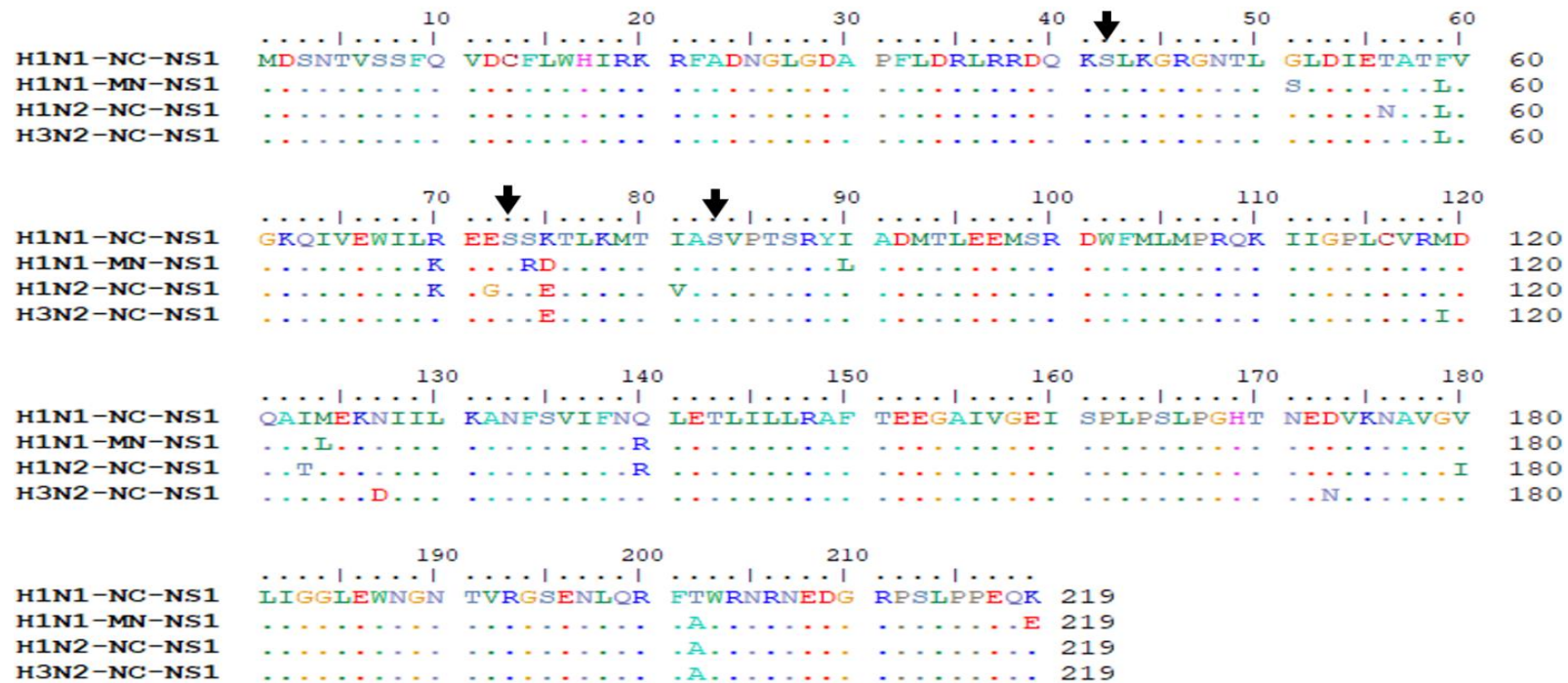
      10      20      30      40      50      60
H1N1-NC-PA-X  . . . . | . . . . | . . . . | . . . . | . . . . | . . . . |
H1N2-NC-PA-X  MEDFVRQCFN PMIVELAEKA MKEYGEDSKI ETNKFAAICT HLEVCFMYSY FHFIDEQGES 60
H3N2-NC-PA-X  MEDFVRQCFN PMIVELAEKA MKEYGEDSKI ETNKFAAICT HLEVCFMYSY FHFIDEQGES 60

      70      80      90     100     110     120
H1N1-NC-PA-X  . . . . | . . . . | . . . . | . . . . | . . . . | . . . . |
H1N2-NC-PA-X  IIVESGDPNA LLKHRFEIIE GRDRIMAWTV VNSICNTTGV EKPKFLPDLY DYKENRFVEI 120
H3N2-NC-PA-X  IIVESGDPNA LLKHRFEIIE GRDRIMAWTV VNSICNTTGV EKPKFLPDLY DYKENRFVEI 120

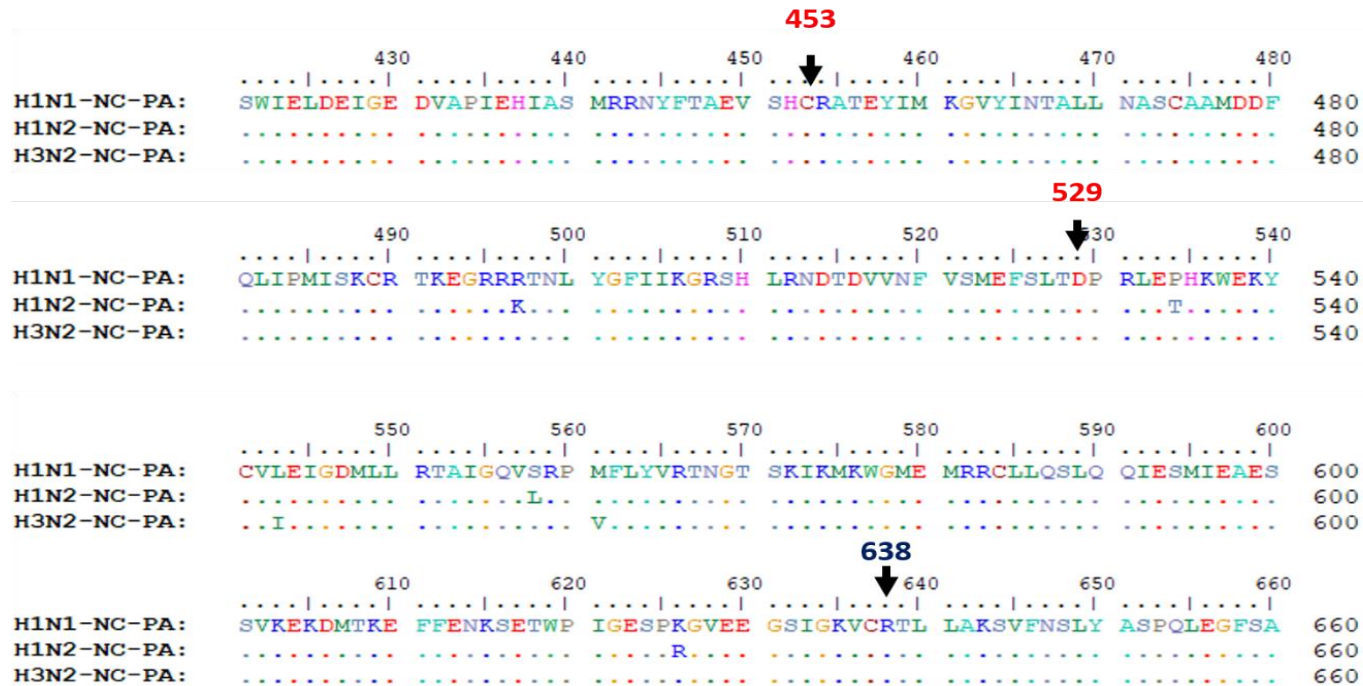
     130     140     150     160     170     180
H1N1-NC-PA-X  . . . . | . . . . | . . . . | . . . . | . . . . | . . . . |
H1N2-NC-PA-X  GVTRREVHHIY YLEKANKIKS EKTHIHIFSF TGEEMATKAD YTLDEESRAR IKTRLFTIRQ 180
H3N2-NC-PA-X  GVTRREVHHIY YLEKANKIKS EKTHIHIFSF TGEEMATKAD YTLDEESRAR IKTRLFTIRQ 180

     190     200     210     220     230
H1N1-NC-PA-X  . . . . | . . . . | . . . . | . . . . | . . . . | . . . . |
H1N2-NC-PA-X  EMASRGLWDS FVSPREAKRQ LKKDLKLQEP CAGLPTKVSH RTSPALKTLE PM 232
H3N2-NC-PA-X  EMASRGLWDS FVSPREAKRQ LKKDLKLQEP CAGLPTKVSH RTSPALKTLE PM 232
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Supplementary Figure 2. Alignment showing NS1 protein sequences between H1N1-NC, H1N1-MN, H1N2-NC, and H3N2-NC viruses. Arrows indicate residues that are critical to inhibition of IFN signaling. Dots indicate identity. Numbers above and to the right indicate position.



Supplementary Figure 3. Alignment showing key residues in PA protein sequences between H1N1-NC, H1N2-NC, and H3N2-NC viruses. Colored numbers indicate residues that contribute to reduced (red) or increased (blue) formation of DI particles. Dots indicate identity. Numbers above and to the right indicate position.



Supplementary Figure 4. Alignment showing differences between PB1-F2 sequences of H1N1-NC, H1N2-NC, and H3N2-NC (a) between H1N1-NC, H1N2-NC PB1-F2, and swine H1 PB1-F2 sequences in the database (b) and H3N2-NC

a)

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      10      20      30      40      50      60
H1N1-NC-PB  MVQPKAWNMM P--LQLHTPG SPRGTALFST QAKG-EFLRM NR--CTRSAA ICLRNF--- 52
H1N2-NC-PB  MVQPKAWNMM P--LQRHTPG SPRGTALFST QAKG-GFLRM NR--CTRSAA ICSRNF--- 52
H3N2-NC-PB  MEQEODIQKK GNGRQIQRLG HPSISIRLMDH YLKIMNQVDM HKQTVSWRPW LSLKNPTQGY 60

      70
H1N1-NC-PB  LVVRTGDRLE F---LAW-- 66
H1N2-NC-PB  LVVRTGDRLE F---LAW-- 66
H3N2-NC-PB  LRIHALKQWK LSNKQGWIN 79
    
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b)

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      10      20      30      40      50      60
H1N1-NC-PB  --MVQPKAWN --MMPLQLHT PGSPRSTALF S----TQAKG EFLRMN---R CTRSAAICLR 49
H1N2-NC-PB  --MVQPKAWN --MMPLQRHT PGSPRSTALF S----TQAKG GFLRMN---R CTRSAIICSR 49
AHB24489.1  -----MLLQLHT PGFPRSTALF S----TQAKG EFLRMN---R CTRSAAIYSR 40
AHB24487.1  MEQEODTPWT QSTEHTNIQK KGNGRQTQRL GHPSSTRLMD HYLKIMNQVD MHRQTVSWRP 60
AHB52159.1  MEQEODTPWT QSTEHTNIQK KGSGRQTQRP GHPSSTRLMD HYLKIMNQVD MHRQTVF--- 57
AIA62030.1  MEQEODTPWT QSTEHTNIQK KGNGRQTQRL GHPSSTRLMD HYLKIMNQVD MHRQTVSWKP 60
AIA61986.1  MEQEODTPWT QSTEHTNIQK KGNGRQTQRL GHPSSTRLMD HYLRTMNQVD MHRQTVFWKP 60
AEH42783.1  MEQEODTPWT QSTEHTNIQK RSGGRQTQRL GHPSSTRLMD HYLKIMNQVD MHRQTVFSRP 60
AEH42772.1  MEQEODTPWT QSTEHTNIQK RSGGRQTQRL GHPSSTRLMD HYLKIMNQVD MHRQTVFSRP 60

      70      80      90      100
H1N1-NC-PB  NFSLVVVRTGD RL---EFLAW ----- 66
H1N2-NC-PB  NFSLVVVRTGD RL---EFLAW ----- 66
AHB24489.1  NFSLVVHTED RL---EFLAW WMPWCLGPGGL MPELTSSLEG LRKKSLLRS 86
AHB24487.1  WLSLKNPTQG YLRIHALKQW KLS--NKQGS----- 87
AHB52159.1  ----- 57
AIA62030.1  WLSLKNPTQG YLRIHALKQW KLS--NKQGW IN----- 90
AIA61986.1  WLSLKSPTQE YLRIHALKQW KLF--NKQGW IN----- 90
AEH42783.1  WLSLKNPTQG YLRIHALKQW KLF--NRQGW IN----- 90
AEH42772.1  WLSLKNPTQG YLRIHALKQW KLF--NRQGW IN----- 90
    
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c)

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      10      20      30      40      50      60
H3N2-NC-PB  MEQEOD--- -----IQ KRNGRQIQR LGHPSISIRLM DHYLKIMNOV DMHRQTVSWR 48
AHB24678.1  MEQEODTP-W TQSTEHTNIQ KRNGRQTQR LGHPSSTRLM DHYLKIMNOV DMHRQTVSWR 59
AHB24678.1  MEQEODTP-W TQSTEHTNIQ KRNGRQTQR LGHPSSTRLM DHYLKIMNOV DMHRQTVSWR 59
AHB23728.1  MEQEODTP-W TQSTEHTNIQ KRNGRQTQR LGHPSSTRLM DHYLKIMNOV DMHRQTVSWR 59
AHB24680.1  MPLQLHTPGF PRGTALFSTQ AKGEFLRMNR CTRSAAT-CS RNFSLVVHTG DRLEFLAWWR 59

      70      80      90
H3N2-NC-PB  PWSLKNPTQ GYLR-IHALK QWKLNSKQGW IN 79
AHB24678.1  PWSLKNPTQ GYLR-IHALK QWKLNSKQGW IN 90
AHB24678.1  PWSLKNPTQ GYLR-IHALK QWKLNSKQGW IN 90
AHB23728.1  PWSLKNPTQ GYLR-IHALK QWKLNSK--- -- 85
AHB24680.1  PWCLGPGIMP GLTSSLDGLR K-KSSLRS-- -- 86
    
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