Supplementary data

Figure S1. Alignment of passage 31 wt, DS and DW IFV B/Maryland/1/59 HA sequences.

B/Maryland/1/59 HA amino acid alignment:

parental strain and passage 31 wt, DS, and DW

```
S136T in DS and DW
G137R in DS and DW
V155I in DW

DS              ----YLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKG 56
DW              -XLIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKG 56
WT              XXIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKG 56
Parental        --------XVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKG 56
                         ***************************************************

DS              TQTRGKLCPNCLNCTDLDVALGRPKCMGTIPSAKVSILHEVKPVTSGCFPIMHDRTKIRQ 110
DW              TQTRGKLCPNCLNCTDLDVALGRPKCMGTIPSAKVSILHEVKPVTSGCFPIMHDRTKIRQ 110
WT              TQTRGKLCPNCLNCTDLDVALGRPKCMGTIPSAKVSILHEVKPVTSGCFPIMHDRTKIRQ 110
Parental        TQTRGKLCPNCLNCTDLDVALGRPKCMGTIPSAKVSILHEVKPVTSGCFPIMHDRTKIRQ 110
                ************************************************************

DS              LPNLLRGYENIRLSTRNVINAETAPGGPYTVGTTRSCPNVTNGKGFFETMAWAVPKNKNK 159
DW              LPNLLRGYENIRLSTRNVINAETAPGGPYTVGTTRSCPNVTNGKGFFETMAWAIPKNKNK 159
WT              LPNLLRGYENIRLSTRNVINAETAPGGPYTVGTSGSCPNVTNGKGFFETMAWAVPKNKNK 159
Parental        LPNLLRGYENIRLSTRNVINAETAPGGPYTVGTSGSCPNVTNGKGFFETMAWAVPKNKNK 159
                *********************************: ******************:******

DS              TATNPLTVEVPYICTKGEDQITVWGFHSDDETQMVILYGDSKPQKFTSSANGVTTHYVSQ 216
DW              TATNPLTVEVPYICTKGEDQITVWGFHSDDETQMVILYGDSKPQKFTSSANGVTTHYVSQ 216
WT              TATNPLTVEVPYICTKGEDQITVWGFHSDDETQMVILYGDSKPQKFTSSANGVTTHYVSQ 216
Parental        TATNPLTVEVPYICTKGEDQITVWGFHSDDETQMVILYGDSKPQKFTSSANGVTTHYVSQ 216
                ************************************************************

DS              IGGFPNQTEDEGLKQSGRIVVDYIVQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSL 272
DW              IGGFPNQTEDEGLKQSGRIVVDYIVQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSL 272
WT              IGGFPNQTEDEGLKQSGRIVVDYIVQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSL 272
Parental        IGGFPNQTEDEGLKQSGRIVVDYIVQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSL 272
                ************************************************************

DS              PLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKER 329
DW              PLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKER 329
WT              PLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKER 329
Parental        PLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKER 329
                ************************************************************

DS              GFFGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEV 389
DW              GFFGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEV 389
WT              GFFGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEV 389
Parental        GFFGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEV 389
                ************************************************************

DS              KNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALER 449
DW              KNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALER 449
WT              KNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALER 449
Parental        KNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALER 449
                ************************************************************

DS              KLKKMLGPSAIDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDD 509
DW              KLKKMLGPSAIDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDD 509
WT              KLKKMLGPSAIDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDD 509
Parental        KLKKMLGPSAIDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAASLNDD 509
                ************************************************************

DS              GLDNHTILLYYSTAASSLAVTLMIAIFIVYMVSRDNVSCSICL 550
DW              GLDNHTILLYYSTAASSLAVTLMIAIFIVYMVSRDNVSCSICL 550
WT              GLDNHTILLYYSTAASSLAVTLMIAIFIVYMVSRDNVSCSICL 550
Parental        GLDNHTILLYYSTAASSLAVTLMIAIFIVYMVSRDNVSCSICL 550
                *******************************************
```

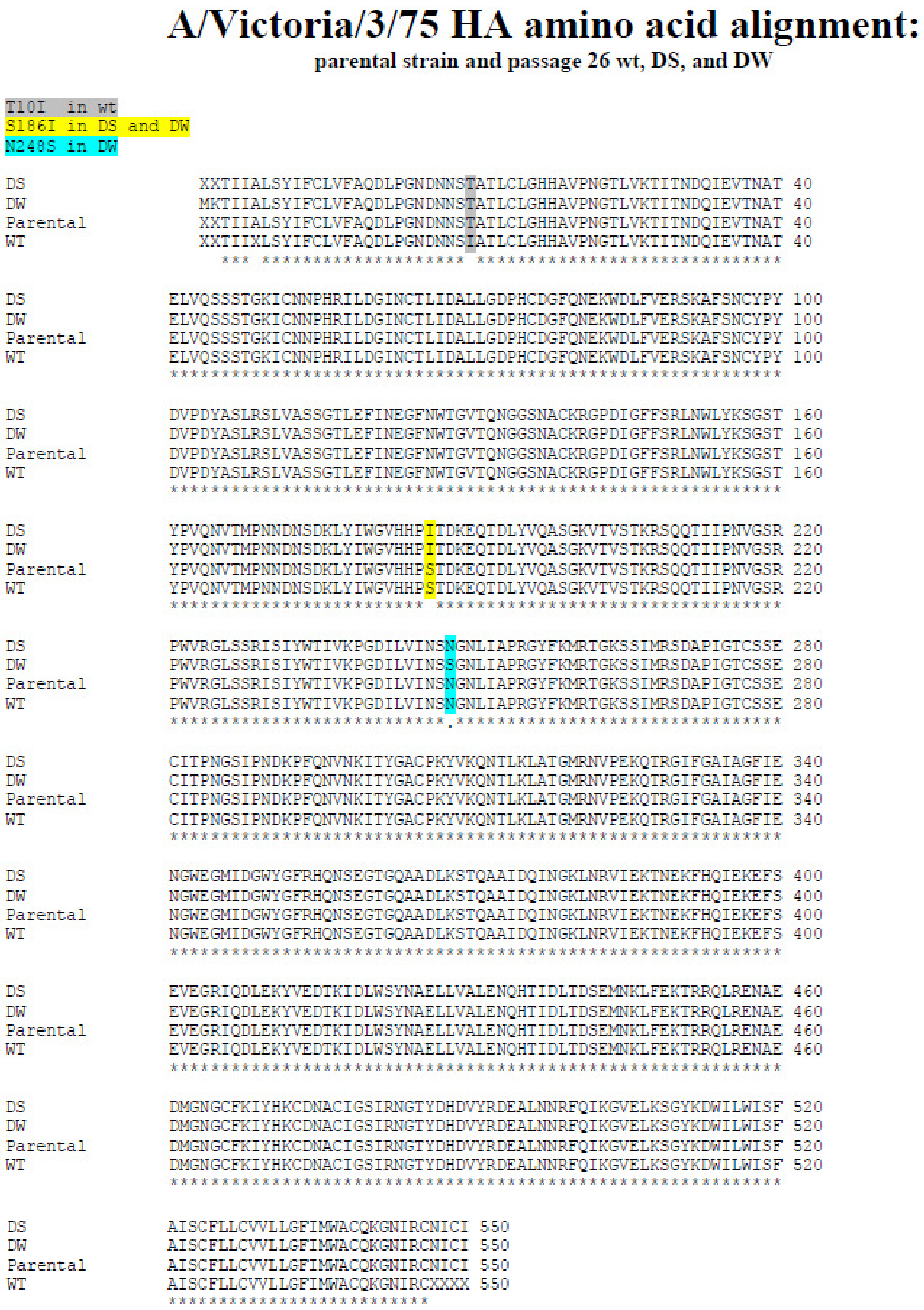
Figure S2. Alignment of passage 31 wt, DS and DW IFV B/Maryland/1/59 NA sequences.

B/Maryland/1/59 NA amino acid alignment:

parental strain and passage 31 wt, DS, and DW

```
S386G in wt
D387G in wt
M467V in wt
L38P  in DS
A268T in DW
W438L in DS and DW


DS              -------------VLLSLYVSASLSYLLYSDILPKFSPTKITAPTMSLECVNVSNAQAVN 63
DW              -------------VLLSLYVSASLSYLLYSDILLKFSPTKITAPTMSLECVNVSNAQAVN 63
Parental        TIQTLTLFLTSGGVLLSLYVSASLSYLLYSDILLKFSPTKITAPTMSLECVNVSNAQAVN 63
WT              TIQTLTLFLTSGGVLLSLYVSASLSYLLYSDILLKFSPTKITAPTMSLECVNVSNAQAVN 63
                             ***********************************************

DS              HSATKEMTFLLPEPEWTYPRLSCQGSTFQKALLISPHRFGETRGNSAPLIIREPFVACGP 126
DW              HSATKEMTFLLPEPEWTYPRLSCQGSTFQKALLISPHRFGETRGNSAPLIIREPFVACGP 126
Parental        HSATKEMTFLLPEPEWTYPRLSCQGSTFQKALLISPHRFGETRGNSAPLIIREPFVACGP 126
WT              HSATKEMTFLLPEPEWTYPRLSCQGSTFQKALLISPHRFGETRGNSAPLIIREPFVACGP 126
                ************************************************************

DS              KECRHFALTHYAAQPGGYYNGTRKDRNKLRHLISVKLGKIPTVENSIFHMAAWSGSACHD 185
DW              KECRHFALTHYAAQPGGYYNGTRKDRNKLRHLISVKLGKIPTVENSIFHMAAWSGSACHD 185
Parental        KECRHFALTHYAAQPGGYYNGTRKDRNKLRHLISVKLGKIPTVENSIFHMAAWSGSACHD 185
WT              KECRHFALTHYAAQPGGYYNGTRKDRNKLRHLISVKLGKIPTVENSIFHMAAWSGSACHD 185
                ************************************************************

DS              GREWTYIGVDGPDSDALIKIKYGEAYTDTYHSYAHNILRTQESACNCIGGDCYLMITDGS 245
DW              GREWTYIGVDGPDSDALIKIKYGEAYTDTYHSYAHNILRTQESACNCIGGDCYLMITDGS 245
Parental        GREWTYIGVDGPDSDALIKIKYGEAYTDTYHSYAHNILRTQESACNCIGGDCYLMITDGS 245
WT              GREWTYIGVDGPDSDALIKIKYGEAYTDTYHSYAHNILRTQESACNCIGGDCYLMITDGS 245
                ************************************************************

DS              ASGISKCRFLKIREGRIIKEIFPAGRVEHTEECTCGFASNKTIECACRDNSYTAKRPFVK 304
DW              ASGISKCRFLKIREGRIIKEIFPTGRVEHTEECTCGFASNKTIECACRDNSYTAKRPFVK 304
Parental        ASGISKCRFLKIREGRIIKEIFPAGRVEHTEECTCGFASNKTIECACRDNSYTAKRPFVK 304
WT              ASGISKCRFLKIREGRIIKEIFPAGRVEHTEECTCGFASNKTIECACRDNSYTAKRPFVK 304
                ***********************:************************************

DS              LNVETDTAEIRLMCTETYLDTPRPDDGSITGPCESNGDKGLGGIKGGFVHQRMASKIGRW 361
DW              LNVETDTAEIRLMCTETYLDTPRPDDGSITGPCESNGDKGLGGIKGGFVHQRMASKIGRW 361
Parental        LNVETDTAEIRLMCTETYLDTPRPDDGSITGPCESNGDKGLGGIKGGFVHQRMASKIGRW 361
WT              LNVETDTAEIRLMCTETYLDTPRPDDGSITGPCESNGDKGLGGIKGGFVHQRMASKIGRW 361
                ************************************************************

DS              YSRTMSKTERMGMELYVKYDGDPWTDSDALAPSGVMVSMKEPGWYSFGFEIKDKKCDVPC 421
DW              YSRTMSKTERMGMELYVKYDGDPWTDSDALAPSGVMVSMKEPGWYSFGFEIKDKKCDVPC 421
Parental        YSRTMSKTERMGMELYVKYDGDPWTDSDALAPSGVMVSMKEPGWYSFGFEIKDKKCDVPC 421
WT              YSRTMSKTERMGMELYVKYDGDPWTDGGALAPSGVMVSMKEPGWYSFGFEIKDKKCDVPC 421
                **************************..********************************

DS              IGIEMVHDGGKETLHSAATAIYCLMGSGQLLWDTVTGVDMAL 469
DW              IGIEMVHDGGKETLHSAATAIYCLMGSGQLLWDTVTGVDMAL 469
Parental        IGIEMVHDGGKETWHSAATAIYCLMGSGQLLWDTVTGVDMAL 469
WT              IGIEMVHDGGKETWHSAATAIYCLMGSGQLLWDTVTGVDVAL 469
                ************* *************************:**
```

Figure S3. Alignment of passage 26 wt, DS and DW IFV A/Victoria/3/75 HA sequences.

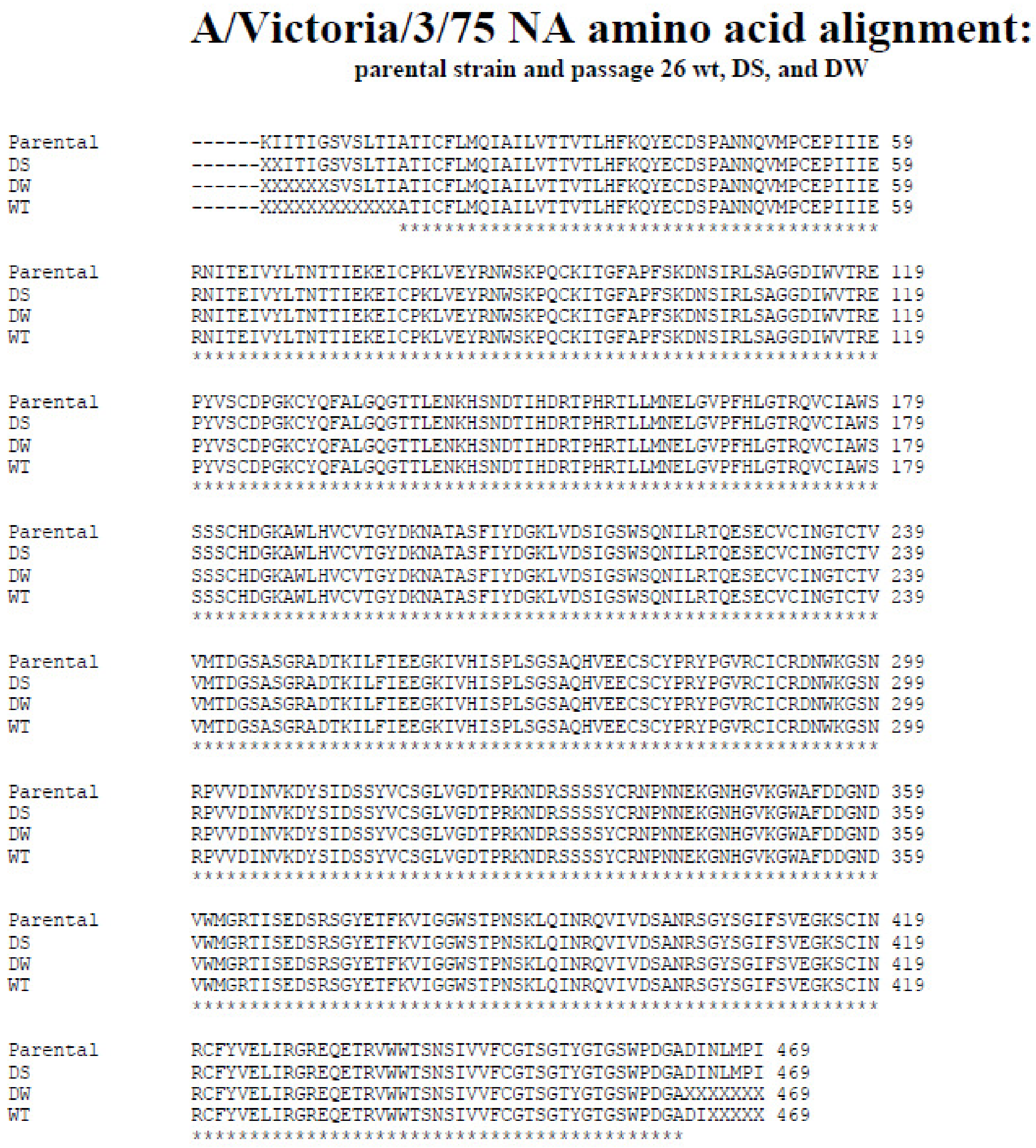


A/Victoria/3/75 HA amino acid alignment:

parental strain and passage 26 wt, DS, and DW

```
T10I  in wt
S186I in DS and DW
N248S in DW

DS                 XXTIIALSYIFCLVFAQDLPGNDNNSTATLCLGHHAVPNGTLVKTITNDQIEVTNAT 40
DW                 MKTIIALSYIFCLVFAQDLPGNDNNSTATLCLGHHAVPNGTLVKTITNDQIEVTNAT 40
Parental           XXTIIALSYIFCLVFAQDLPGNDNNSTATLCLGHHAVPNGTLVKTITNDQIEVTNAT 40
WT                 XXTIIXLSYIFCLVFAQDLPGNDNNSIATLCLGHHAVPNGTLVKTITNDQIEVTNAT 40
                     *** ******************** ******************************

DS              ELVQSSSTGKICNNPHRILDGINCTLIDALLGDPHCDGFQNEKWDLFVERSKAFSNCYPY 100
DW              ELVQSSSTGKICNNPHRILDGINCTLIDALLGDPHCDGFQNEKWDLFVERSKAFSNCYPY 100
Parental        ELVQSSSTGKICNNPHRILDGINCTLIDALLGDPHCDGFQNEKWDLFVERSKAFSNCYPY 100
WT              ELVQSSSTGKICNNPHRILDGINCTLIDALLGDPHCDGFQNEKWDLFVERSKAFSNCYPY 100
                ************************************************************

DS              DVPDYASLRSLVASSGTLEFINEGFNWTGVTQNGGSNACKRGPDIGFFSRLNWLYKSGST 160
DW              DVPDYASLRSLVASSGTLEFINEGFNWTGVTQNGGSNACKRGPDIGFFSRLNWLYKSGST 160
Parental        DVPDYASLRSLVASSGTLEFINEGFNWTGVTQNGGSNACKRGPDIGFFSRLNWLYKSGST 160
WT              DVPDYASLRSLVASSGTLEFINEGFNWTGVTQNGGSNACKRGPDIGFFSRLNWLYKSGST 160
                ************************************************************

DS              YPVQNVTMPNNDNSDKLYIWGVHHPITDKEQTDLYVQASGKVTVSTKRSQQTIIPNVGSR 220
DW              YPVQNVTMPNNDNSDKLYIWGVHHPITDKEQTDLYVQASGKVTVSTKRSQQTIIPNVGSR 220
Parental        YPVQNVTMPNNDNSDKLYIWGVHHPSTDKEQTDLYVQASGKVTVSTKRSQQTIIPNVGSR 220
WT              YPVQNVTMPNNDNSDKLYIWGVHHPSTDKEQTDLYVQASGKVTVSTKRSQQTIIPNVGSR 220
                ************************* **********************************

DS              PWVRGLSSRISIYWTIVKPGDILVINSNGNLIAPRGYFKMRTGKSSIMRSDAPIGTCSSE 280
DW              PWVRGLSSRISIYWTIVKPGDILVINSSGNLIAPRGYFKMRTGKSSIMRSDAPIGTCSSE 280
Parental        PWVRGLSSRISIYWTIVKPGDILVINSNGNLIAPRGYFKMRTGKSSIMRSDAPIGTCSSE 280
WT              PWVRGLSSRISIYWTIVKPGDILVINSNGNLIAPRGYFKMRTGKSSIMRSDAPIGTCSSE 280
                ***************************.********************************

DS              CITPNGSIPNDKPFQNVNKITYGACPKYVKQNTLKLATGMRNVPEKQTRGIFGAIAGFIE 340
DW              CITPNGSIPNDKPFQNVNKITYGACPKYVKQNTLKLATGMRNVPEKQTRGIFGAIAGFIE 340
Parental        CITPNGSIPNDKPFQNVNKITYGACPKYVKQNTLKLATGMRNVPEKQTRGIFGAIAGFIE 340
WT              CITPNGSIPNDKPFQNVNKITYGACPKYVKQNTLKLATGMRNVPEKQTRGIFGAIAGFIE 340
                ************************************************************

DS              NGWEGMIDGWYGFRHQNSEGTGQAADLKSTQAAIDQINGKLNRVIEKTNEKFHQIEKEFS 400
DW              NGWEGMIDGWYGFRHQNSEGTGQAADLKSTQAAIDQINGKLNRVIEKTNEKFHQIEKEFS 400
Parental        NGWEGMIDGWYGFRHQNSEGTGQAADLKSTQAAIDQINGKLNRVIEKTNEKFHQIEKEFS 400
WT              NGWEGMIDGWYGFRHQNSEGTGQAADLKSTQAAIDQINGKLNRVIEKTNEKFHQIEKEFS 400
                ************************************************************

DS              EVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTRRQLRENAE 460
DW              EVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTRRQLRENAE 460
Parental        EVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTRRQLRENAE 460
WT              EVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTRRQLRENAE 460
                ************************************************************

DS              DMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISF 520
DW              DMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISF 520
Parental        DMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISF 520
WT              DMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISF 520
                ************************************************************

DS              AISCFLLCVVLLGFIMWACQKGNIRCNICI 550
DW              AISCFLLCVVLLGFIMWACQKGNIRCNICI 550
Parental        AISCFLLCVVLLGFIMWACQKGNIRCNICI 550
WT              AISCFLLCVVLLGFIMWACQKGNIRCXXXX 550
                **************************
```

Figure S4. Alignment of passage 26 wt, DS and DW IFV A/Victoria/3/75 NA sequences.

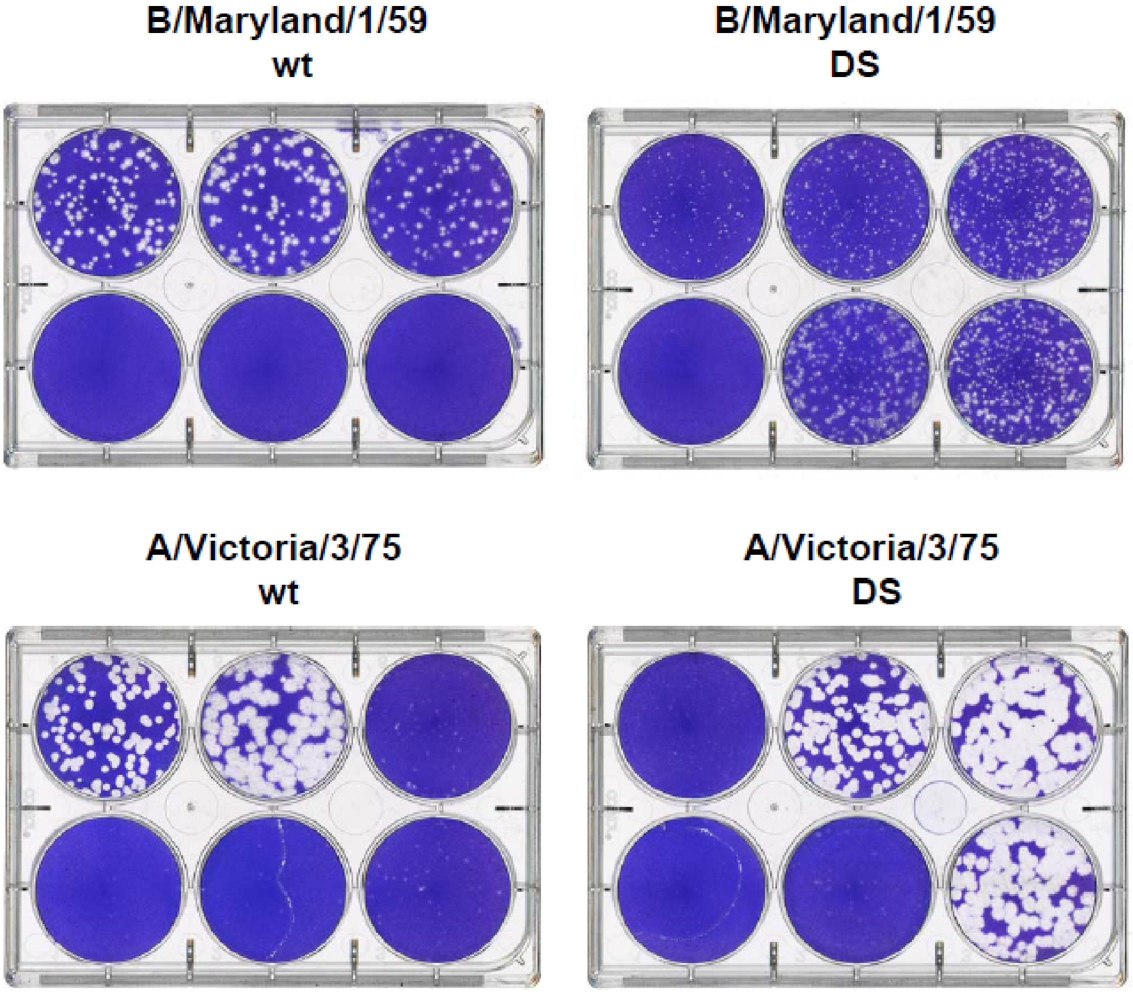


A/Victoria/3/75 NA amino acid alignment:

parental strain and passage 26 wt, DS, and DW

```
Parental        ------KIITIGSVSLTIATICFLMQIAILVTTVTLHFKQYECDSPANNQVMPCEPIIIE 59
DS              ------XXITIGSVSLTIATICFLMQIAILVTTVTLHFKQYECDSPANNQVMPCEPIIIE 59
DW              ------XXXXXXSVSLTIATICFLMQIAILVTTVTLHFKQYECDSPANNQVMPCEPIIIE 59
WT              ------XXXXXXXXXXXXATICFLMQIAILVTTVTLHFKQYECDSPANNQVMPCEPIIIE 59
                                  ******************************************

Parental        RNITEIVYLTNTTIEKEICPKLVEYRNWSKPQCKITGFAPFSKDNSIRLSAGGDIWVTRE 119
DS              RNITEIVYLTNTTIEKEICPKLVEYRNWSKPQCKITGFAPFSKDNSIRLSAGGDIWVTRE 119
DW              RNITEIVYLTNTTIEKEICPKLVEYRNWSKPQCKITGFAPFSKDNSIRLSAGGDIWVTRE 119
WT              RNITEIVYLTNTTIEKEICPKLVEYRNWSKPQCKITGFAPFSKDNSIRLSAGGDIWVTRE 119
                ************************************************************

Parental        PYVSCDPGKCYQFALGQGTTLENKHSNDTIHDRTPHRTLLMNELGVPFHLGTRQVCIAWS 179
DS              PYVSCDPGKCYQFALGQGTTLENKHSNDTIHDRTPHRTLLMNELGVPFHLGTRQVCIAWS 179
DW              PYVSCDPGKCYQFALGQGTTLENKHSNDTIHDRTPHRTLLMNELGVPFHLGTRQVCIAWS 179
WT              PYVSCDPGKCYQFALGQGTTLENKHSNDTIHDRTPHRTLLMNELGVPFHLGTRQVCIAWS 179
                ************************************************************

Parental        SSSCHDGKAWLHVCVTGYDKNATASFIYDGKLVDSIGSWSQNILRTQESECVCINGTCTV 239
DS              SSSCHDGKAWLHVCVTGYDKNATASFIYDGKLVDSIGSWSQNILRTQESECVCINGTCTV 239
DW              SSSCHDGKAWLHVCVTGYDKNATASFIYDGKLVDSIGSWSQNILRTQESECVCINGTCTV 239
WT              SSSCHDGKAWLHVCVTGYDKNATASFIYDGKLVDSIGSWSQNILRTQESECVCINGTCTV 239
                ************************************************************

Parental        VMTDGSASGRADTKILFIEEGKIVHISPLSGSAQHVEECSCYPRYPGVRCICRDNWKGSN 299
DS              VMTDGSASGRADTKILFIEEGKIVHISPLSGSAQHVEECSCYPRYPGVRCICRDNWKGSN 299
DW              VMTDGSASGRADTKILFIEEGKIVHISPLSGSAQHVEECSCYPRYPGVRCICRDNWKGSN 299
WT              VMTDGSASGRADTKILFIEEGKIVHISPLSGSAQHVEECSCYPRYPGVRCICRDNWKGSN 299
                ************************************************************

Parental        RPVVDINVKDYSIDSSYVCSGLVGDTPRKNDRSSSSYCRNPNNEKGNHGVKGWAFDDGND 359
DS              RPVVDINVKDYSIDSSYVCSGLVGDTPRKNDRSSSSYCRNPNNEKGNHGVKGWAFDDGND 359
DW              RPVVDINVKDYSIDSSYVCSGLVGDTPRKNDRSSSSYCRNPNNEKGNHGVKGWAFDDGND 359
WT              RPVVDINVKDYSIDSSYVCSGLVGDTPRKNDRSSSSYCRNPNNEKGNHGVKGWAFDDGND 359
                ************************************************************

Parental        VWMGRTISEDSRSGYETFKVIGGWSTPNSKLQINRQVIVDSANRSGYSGIFSVEGKSCIN 419
DS              VWMGRTISEDSRSGYETFKVIGGWSTPNSKLQINRQVIVDSANRSGYSGIFSVEGKSCIN 419
DW              VWMGRTISEDSRSGYETFKVIGGWSTPNSKLQINRQVIVDSANRSGYSGIFSVEGKSCIN 419
WT              VWMGRTISEDSRSGYETFKVIGGWSTPNSKLQINRQVIVDSANRSGYSGIFSVEGKSCIN 419
                ************************************************************

Parental        RCFYVELIRGREQETRVWWTSNSIVVFCGTSGTYGTGSWPDGADINLMPI 469
DS              RCFYVELIRGREQETRVWWTSNSIVVFCGTSGTYGTGSWPDGADINLMPI 469
DW              RCFYVELIRGREQETRVWWTSNSIVVFCGTSGTYGTGSWPDGAXXXXXXX 469
WT              RCFYVELIRGREQETRVWWTSNSIVVFCGTSGTYGTGSWPDGADIXXXXX 469
                *******************************************
```

Figure S5. DS IFV in plaque assay. Confluent monolayers of MDCK cells were infected with wt and DS IFVs and overlaid with 1:1 agar/medium to create plaques. Indicated viruses were applied to each well at equivalent pfu (150 pfu/well) and DAS181 was included in the agar/medium solution. Increasing concentrations of DAS181 were used (from upper left-hand corner of each plate going clockwise: 0, 0.0015, 0.015, 0.15, 1.5 and 15 μM).



B/Maryland/1/59
wt

B/Maryland/1/59
DS

A/Victoria/3/75
wt

A/Victoria/3/75
DS

Table S1. HA and NA mutations identified during IFV B/Maryland/1/59 passaging

Passage #	HA mutations			NA mutations		
	wt	DS	DW	wt	DS	DW
4	–	–		–	–	
5	–	G137R		–	–	
6	–	G137R		–	–	
7	–	G137R		–	–	
8	–	G137R		–	–	
12	–	G137R		–	–	
13	–	G137R, S136T		–	–	
14	–	G137R, S136T		–	–	
18	–	G137R, S136T		–	W438L	
19	–	G137R, S136T	G137R, S136T	–	W438L	W438L
22	V247M	G137R, S136T	G137R, S136T, deletions[a]	S386G[a], D387G[a]	W438L	W438L, deletions[a]
23	V247M	G137R, S136T	G137R, S136T, deletions[a], V155I	S386G[a], D387G[a]	W438L	W438L, deletions[a]
27	V247M	G137R, S136T	G137R, S136T, V155I	S386G[a], D387G[a]	W438L[a], L38P[a]	W438L, A268T[a]
29	–	G137R, S136T	G137R, S136T, V155I	S386G[a], D387G[a]	W438L[a], L38P[a]	W438L, A268T
34	–	G137R, S136T	G137R, S136T, V155I	S386G[a], D387G[a]	W438L[a], L38P[a]	W438L, A268T

–, No mutations observed.

[a] Sequence heterogeneity observed for this amino acid.

HA and NA sequences from wt, DS and DW IFV B/Maryland/1/59 were compared with initial viral stock sequence at most passages.

Mutations observed at select/representative passages are shown.

B/Maryland/1/59 DW virus was initiated at passage 17 and is thus only described beyond this point.

Sequence data noted with H3 and N2 numbering schemes, as previously described.[21,22]

Full sequence alignments are shown in Figures S1 and S2.

Table S2. HA and NA mutations identified during IFV A/Victoria/3/75 passaging

Passage #	HA mutations			NA mutations		
	wt	DS	DW	wt	DS	DW
1	–	–		–	–	
2	–	–		–	–	
3	–	–		–	–	
4	–	N126T, E325K		–	–	
5	–	N126T		–	–	
6	–	S186I		–	–	
12	–	S186I		–	–	
14	–	S186I		–	K210R	
15	–	S186I	S186I	–	–	K210R[a]
17	T10I[a]	S186I	S186I, K68R[b]	–	–	K210R[a]
19	T10I	S186I	S186I, K68R[b], N248S[a]	–	–	K210R[a]
21	T10I	S186I	S186I, K68R[b], N248S	–	–	–
24	T10I	S186I	S186I, N248S	–	–	–
26	T10I	S186I	S186I, N248S	–	–	–

–, No mutations observed.

[a] Sequence heterogeneity observed for this amino acid.

[b] Mutation lies in HA2 segment, all others in HA1 segment.

HA and NA sequences from wt, DS and DW IFV A/Victoria/3/75 were compared with initial viral stock sequence at most passages.

Mutations observed at select/representative passages are shown.

AVictoria/3/75 DW virus was initiated at passage 14 and is thus only described beyond this point.

Sequence data noted with H3 and N2 numbering schemes, as previously described.[21,22]

Full sequence alignments are shown in Figures S3 and S4.

Table S3. Top 20 sialylated glycan structures bound on microarray by wt and DS IFV B/Maryland/1/59

Glycan #	Glycan structure	RFU
B/Maryland/1/59/wt		
361	Neu5Acα2-6GlcNAcβ1-4GlcNAc-Sp21	30797
362	Neu5Acα2-6GlcNAcβ1-4GlcNAcβ1-4GlcNAc-Sp21	16440
318	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-N(LT)AVL	3385
326	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0	2157
244	Neu5Acα2-6Galβ1-4GlcNAcβ-Sp8	1988
246	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0	1836
324	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-3GlcNAcβ-Sp0	1300
242	Neu5Acα2-6Galβ1-4[6OSO3]GlcNAcβ-Sp8	1032
198	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	912
214	Neu5Acα2-3GalNAcβ1-4GlcNAcβ-Sp0	901
340	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc-Sp12	880
241	Neu5Acα2-6GalNAcβ1-4GlcNAcβ-Sp0	839
315	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	831
282	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-3GlcNAcβ-Sp0	803
237	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0	732
342	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3Manβ1-4GlcNAcβ1-4GlcNAc-Sp12	689
53	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp13	641
52	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	587
371	NeuAcα2-3Galβ1-4GlcNAcβ1-3GalNAc-Sp14	528
243	Neu5Acα2-6Galβ1-4GlcNAcβ-Sp0	512
B/Maryland/1/59/DS		
315	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	3700
214	Neu5Acα2-3GalNAcβ1-4GlcNAcβ-Sp0	3205
237	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0	3078
373	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3GalNAcα-Sp14	2773
371	NeuAcα2-3Galβ1-4GlcNAcβ1-3GalNAc-Sp14	2655
282	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-3GlcNAcβ-Sp0	2553
234	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp0	2552

142	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	2442
236	Neu5Acα2-3Galβ1-4GlcNAcβ-Sp8	2201
361	Neu5Acα2-6GlcNAcβ1-4GlcNAc-Sp21	2016
312	Neu5Acα2-3Galβ1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-6)GalNAcα-Sp14	1917
231	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ-Sp8	1850
198	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Sp12	1802
218	Neu5Acα2-3Galβ1-3(Neu5Acα2-3Galβ1-4)GlcNAcβ-Sp8	1796
225	Neu5Acα2-3Galβ1-3GlcNAcβ-Sp8	1787
318	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-N(LT)AVL	1769
238	Neu5Acα2-3Galβ1-4Glcβ-Sp0	1698
235	Neu5Acα2-3Galβ1-4GlcNAcβ-Sp0	1695
232	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4GlcNAcβ-Sp8	1662
224	Neu5Acα2-3Galβ1-3GlcNAcβ-Sp0	1634

Information for the top 20 glycans bound by wt and DS IFV B/Marlyland/1/59 (from graphs shown in Figure 9) is listed as the glycan number, the shorthand chemical name/structure and the RFU observed.