

Tutorial:
How to do tentative clade
assignments of seasonal H3 and
H1pdm in GISAID using FluServer
and NEXTFLU



GISAIID EpiFlu - Global In x

platform.gisaid.org/epi3/frontend#2e7a86

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GISAIID

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Count GISAID published Total isolate count

Basic filters

Predefined search

Search in Released files Worksets

Search patterns

Type	H	N	Lineage	Host	Location
A	1	1	unknown	-all-	China
B	2	2	pdm09	Human	Christmas Island
C	3	3	seasonal	Animal	Georgia
	4	4		Avian	Hong Kong (SAR)
	5	5		Chicken	India

Additional filters

Collection date (YYYY-MM-DD) From To

Submission date (YYYY-MM-DD) From To

Originating Laboratory

Submitting Laboratory

Required Segments PB2 PB1 PA HA NP NA MP NS HE P3

full genome only complete Min Length

only GISAID uploaded isolates only INSDC imported isolates

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Select the strains of interest in GISAID

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Released files

<input checked="" type="checkbox"/>	edit	Name	Isolate ID	Subtype	Host	Collection date	Passage	PB2	PB1	PA	HA	NP	NA	MP	NS
<input checked="" type="checkbox"/>		A/India/6012/2017	EPI_ISL_295987	H1N1	Human	2017-10-18	X1	2316	2316	2208	1752	1540	1433	1002	865
<input checked="" type="checkbox"/>		A/India/1038/2017	EPI_ISL_295988	H1N1	Human	2017-12-20	X2	2316	2316	2208	1752	1540	1433	1002	865
<input checked="" type="checkbox"/>		A/India/5889/2017	EPI_ISL_295988	H1N1	Human	2017-10-13	X1	2316	2316	2208	1752	1540	1433	1002	865
<input checked="" type="checkbox"/>		A/India/9929/2017	EPI_ISL_295984	H1N1	Human	2017-10-25	X1	2316	2316	2208	1752	1540	1433	1002	865

Total: 4 isolates << first < prev 1 next > last >>

Search in results

Go back Help Copy to... Add to analysis Download

Select the strains and add to the analysis



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Released files

<input type="checkbox"/>	edit	Name
<input type="checkbox"/>		A/India/6012/2
<input type="checkbox"/>		A/India/1038/2
<input type="checkbox"/>		A/India/5869/2
<input type="checkbox"/>		A/India/9929/2

	NA	MP
	1433	1002
	1433	1002
	1433	1002
	1433	1002

Choose analysis

Alignment
Align DNA or Proteins

List of third party servers

FluSurver
FluSurver



Choose FluSurver as analysis tool

Total: 4 isolates

Search in results



FluSurfer

Filter Align DNA (NC) Align Proteins (AA)
 all PB2 PB1 PA HA NP NA MP NS HE P3

<input type="checkbox"/>	Sequence name	EPI/WSS-Nr	Isolate name	NCBI-Id	Length
<input checked="" type="checkbox"/>	3000883447_N8K8Y7CT_v1_4	EPI1162738	A/India/8012/2017		1752
<input checked="" type="checkbox"/>	3000883448_N8K8Y7CY_v1_4	EPI1161985	A/India/8889/2017		1752
<input checked="" type="checkbox"/>	3000886516_N8K8YJ72_v1_4	EPI1161934	A/India/9829/2017		1752
<input checked="" type="checkbox"/>	3000886518_N8K8YJ7C_v1_4	EPI1164289	A/India/1038/2017		1752

Choose the segment/protein to use
(HA for clade assignment!)



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Important usage notes:

The main application scenario for FluSurver is to highlight phenotypically or epidemiologically interesting candidate mutations for further research and should ideally be combined with experimental testing and verification of any predicted phenotypes. Importantly, any direct diagnostic use, assumed severity or recommendation on patient treatment should not be based solely on these computational predictions. Our curated reference sequences used for annotation transfer of equivalent mutations are mainly comprised of strains that recently infected humans. Therefore, the **usage scenario that will give the most fruitful and reliable results are current surveillance sequences with very close relation to used vaccine strains, including some candidates for avian flu (including the recent H7N9 and avian H5N8, for GISAID users only!) and novel reassortant swine flu H3N2v.** Please take a look at the [Frequently Asked Questions](#) and [Tutorial](#) if you are new to FluSurver. You could also look at this [NA example analysis walkthrough](#) and the [GISAID access preview poster](#).

Note for **H7N9** analysis: A tutorial with example analysis and interpretation is available [here](#).

Loaded protein sequences of your selected isolates for FluSurver analysis:

```
>HA_A/India/1038/2017_295986
MKAILVLLYFTTTANADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNGLCKLRGVAPLHLGKCNIAAGWILGNPECESLSTARSWSYIVETSNSDNGTC
YPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLKKGNSYPKLNQTYINDKGKEVLVLWGIHHPSTADQQSLYQNADA
YVFGTSRYSKFKFPEIATRPKVRDQEGRMNYWTLVPEPDKITFEATGNLVVPRYAFTMERNAGSGIISDTPVHDCNTTCQTPEGAINTSLPFQNVHPITIGKCP
KYVVKSTKLRLATGLRNVPISQSRGLFGAIAGFIEGGWTGMVDGWIYGYHHQNEQSSGYAADLKSQTQNAVDKITNKVNSVIEKMMTQFVAVGKEFNHLEKRIENLNKKV
DDGFLDIWYNAELLVLL ENERTLDYHDSNVKNLYEKVRNQLKNAKEINGNCFEFYHKDNTCMESVKNGTVDYPKYSEEAKLNREKIDGVKLESTRVYQILAIYS
TVASSLVLVSLGAISFWMCSNGSLQCRICI
>HA_A/India/5869/2017_295888
MKAILVLLYFTTTANADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLLEDKHNGLCKLRGVAPLHLGKCNIAAGWILGNPECESLSTARSWSYIVETSNSDNGTC
YPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAAACPHAGAKSFYKNLIWLKKGNSYPKLNQTYINDKGKEVLVLWGIHHPSTADQQSLYQNADA
```

Please select the reference strain(s) to compare to:

Automatic detection of closest reference (larger selection of strains, not always full genomes, NOT SUITED to judge reassortment) ▾

Analyze with FluSurver

← Click Analyze!



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Result for comparison with reference selection: autorefall [Back to Reference Selection](#)

Query	Best reference hit	% AA identity	% length coverage	# mutations	List of mutations
HA_A/India/1038/2017_295986	HA A/Michigan/45/2015(H1N1) find closest related sequences	98.940	100.000	6	S91R , S181T , R240Q , I312V , I389V , I527V show in structure
HA_A/India/5869/2017_295888	HA A/Michigan/45/2015(H1N1) find closest related sequences	99.117	100.000	5	S91R , S181T , S200P , R240Q , I312V show in structure
HA_A/India/6012/2017_295987	HA A/Michigan/45/2015(H1N1) find closest related sequences	98.940	100.000	6	S91R , N146D , S181T , S200P , R240Q , I312V show in structure
HA_A/India/9929/2017_295884	HA A/Michigan/45/2015(H1N1) find closest related sequences	99.293	100.000	4	S91R , S181T , R240Q , I312V show in structure

[Right-click here to save/download mutation report table for archiving or import to Excel](#)

Warning: this reference selection includes sequences of strains without complete genomes, e.g. only HA and NA available included. Therefore, hits of other segments to different reference strains do not represent reassortments. Please see also [this help section](#) regarding reassortments.

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S91R, S181T, R240Q, I312V are shared among the samples, we can use these mutations to see if they are linked to specific named clades by connecting with NextFlu

The main application scenario for FluSurver is to highlight phenotypically or epidemiologically interesting candidate mutations for further research and should ideally be combined with experimental testing and verification of any predicted phenotypes. Importantly, any direct diagnostic use, assumed severity or recommendation on patient treatment should not be based solely on these computational predictions. Our curated reference sequences used for annotation transfer of equivalent mutations are mainly comprised of strains that recently infected humans. Therefore, **the usage scenario that will give the most fruitful and reliable results are current surveillance sequences with very close relation to used vaccine strains, including some candidates for avian flu and novel reassortant swine flu H3N2v.** Please take a look at the [Frequently Asked Questions](#) and [Tutorial](#) if you are new to FluSurver. There is also a [special note for using FluSurver results in publications](#).



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[Right-click here to save/download mutation report table for archiving or import to Excel](#)

Warning: this reference selection includes sequences of strains without complete genomes, e.g. only HA and NA available/included. Therefore, hits of other segments to different reference strains do not represent reassortments. Please see also [this help section](#) regarding reassortments.

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Click at one of the possible clade-linked mutations

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The main application scenario for FluSurver is to highlight phenotypically or epidemiologically interesting candidate mutations for further research and should ideally be combined with experimental testing and verification of any predicted phenotypes. Importantly, any direct diagnostic use, assumed severity or recommendation on patient treatment should not be based solely on these computational predictions. Our curated reference sequences used for annotation transfer of equivalent mutations are mainly comprised of strains that recently infected humans. Therefore, the usage scenario that will give the most fruitful and reliable results are current surveillance.

HA S91R
 Key to alternative position numbering:
 FluSurver numbering (absolute as in 2009 H1N1 pandemic)
 91
 Classical H3N2 strain numbering
 HA1 82
 Classical H1N1 strain numbering
HA1 74

Chosen reference: HA_H1N1_Human_2015_Michigan45
 Position in reference: 91
 AA in reference: S
 AA in query: R

Mutation HA S91R already occurred 9 times (0.38% of all samples with HA sequence) in 2 countries. The first strain with this mutation, collected in December 2015, was A/Shiraz/106/2015. The mutation most recently occurred in strain A/Virginia/23/2017, collected in March 2017. ([see map](#))
[See detailed global statistics for this position](#)

As seen in resolved structures of proteins from related strains, the HA position equivalent to your mutation is involved in:

- [viral oligomerization interfaces](#)
- [binding small ligand\(s\)](#)
- [antibody recognition sites](#)

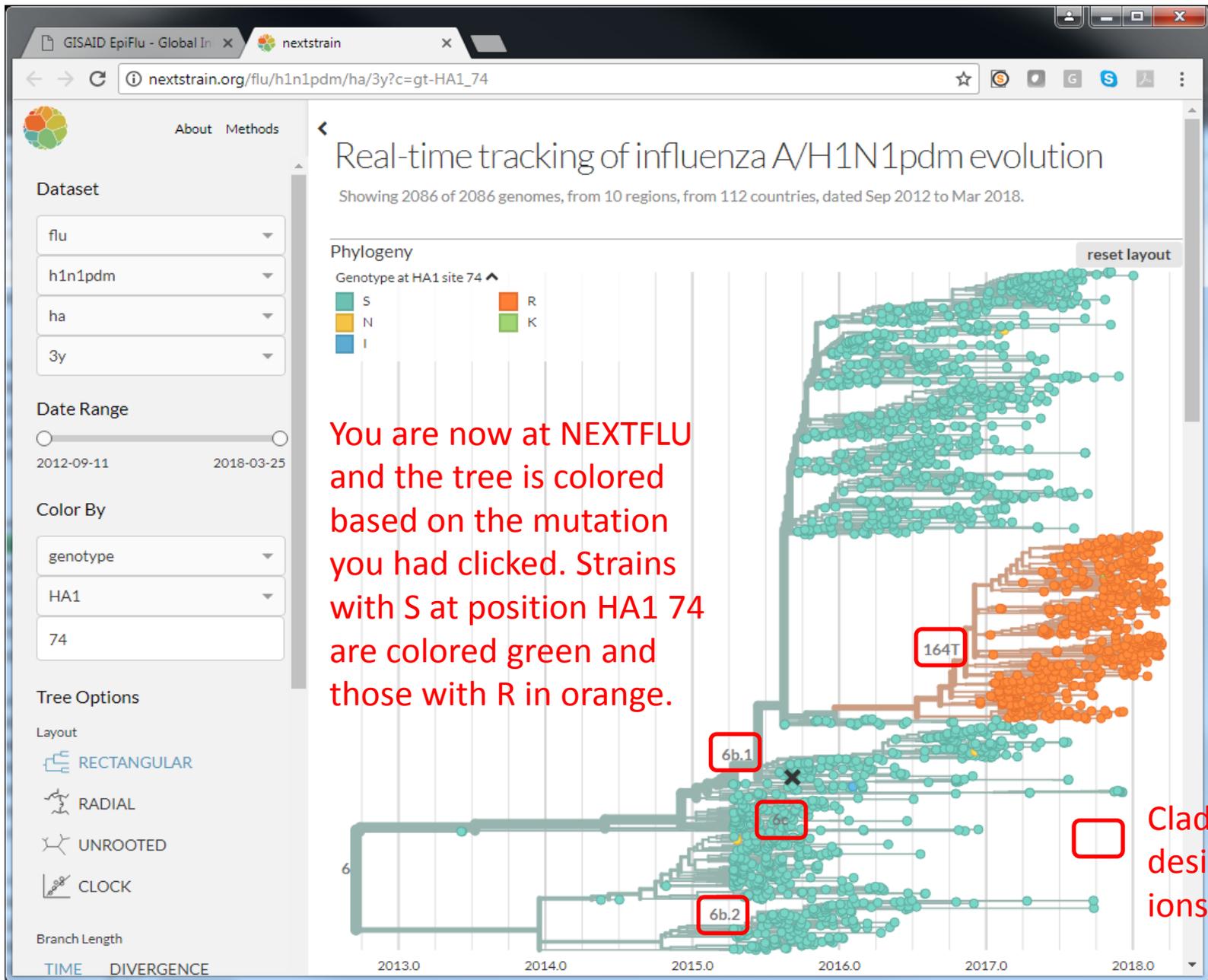
[See all interactions for this position](#)

[PubMed search for this mutation \(including alternative numbering\)](#)
 NEW: [Phylogenetic context for this mutation at NEXTFLU](#)



[Go back](#)

See the alternative numbering for your information (HA1 74 without signal peptide) and click on link to NEXTFLU



You are now at NEXTFLU and the tree is colored based on the mutation you had clicked. Strains with S at position HA1 74 are colored green and those with R in orange.

Clade designations

The orange S91R/S74R strains branched off clade 6b.1 and include clade 164T

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HA S181T

Key to alternative position numbering:

181	FluSurver numbering (absolute as in 2009 H1N1 pandemic)
HA1 167	Classical H3N2 strain numbering
HA1 164	Classical H1N1 strain numbering

Chosen reference: HA_H1N1_Human_2015_Michigan45

Position in reference: 181
 AA in reference: S
 AA in query: T

Mutation HA S181T already occurred 2 time(s) (0.08% of all samples with HA sequence) in 1 country. The first strain with this mutation, collected in March 2016, was A/Massachusetts/5373/2016. The mutation most recently occurred in strain A/Utah/37/2016, collected in May 2016. ([see map](#))
[See detailed global statistics for this position](#)

As seen in resolved structures of proteins from related strains, the HA position equivalent to your mutation is involved in:

- [binding small ligand\(s\)](#)
- [viral oligomerization interfaces](#)
- [antibody recognition sites](#)

[See all interactions for this position](#)

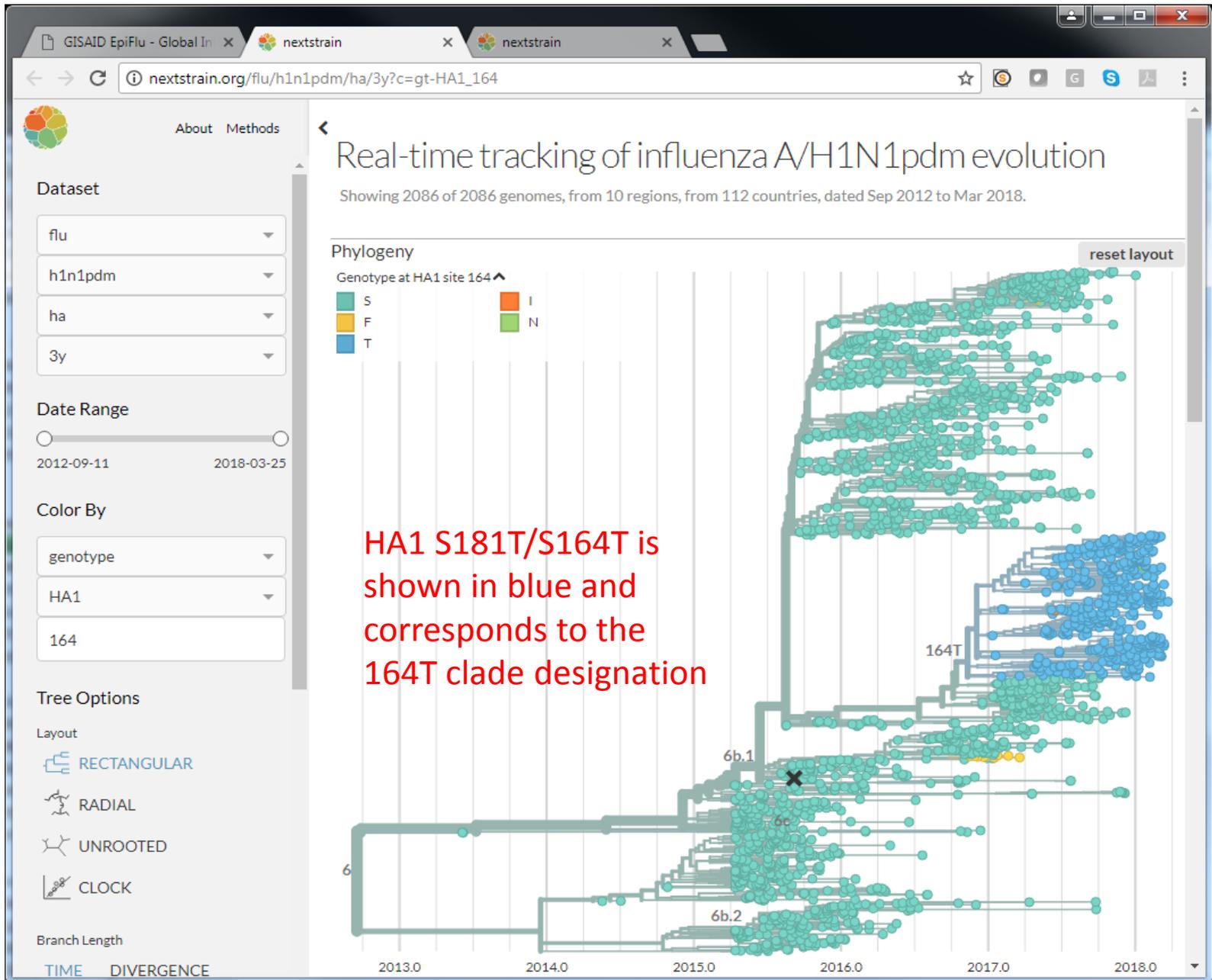
[PubMed search for this mutation \(including alternative numbering\)](#)

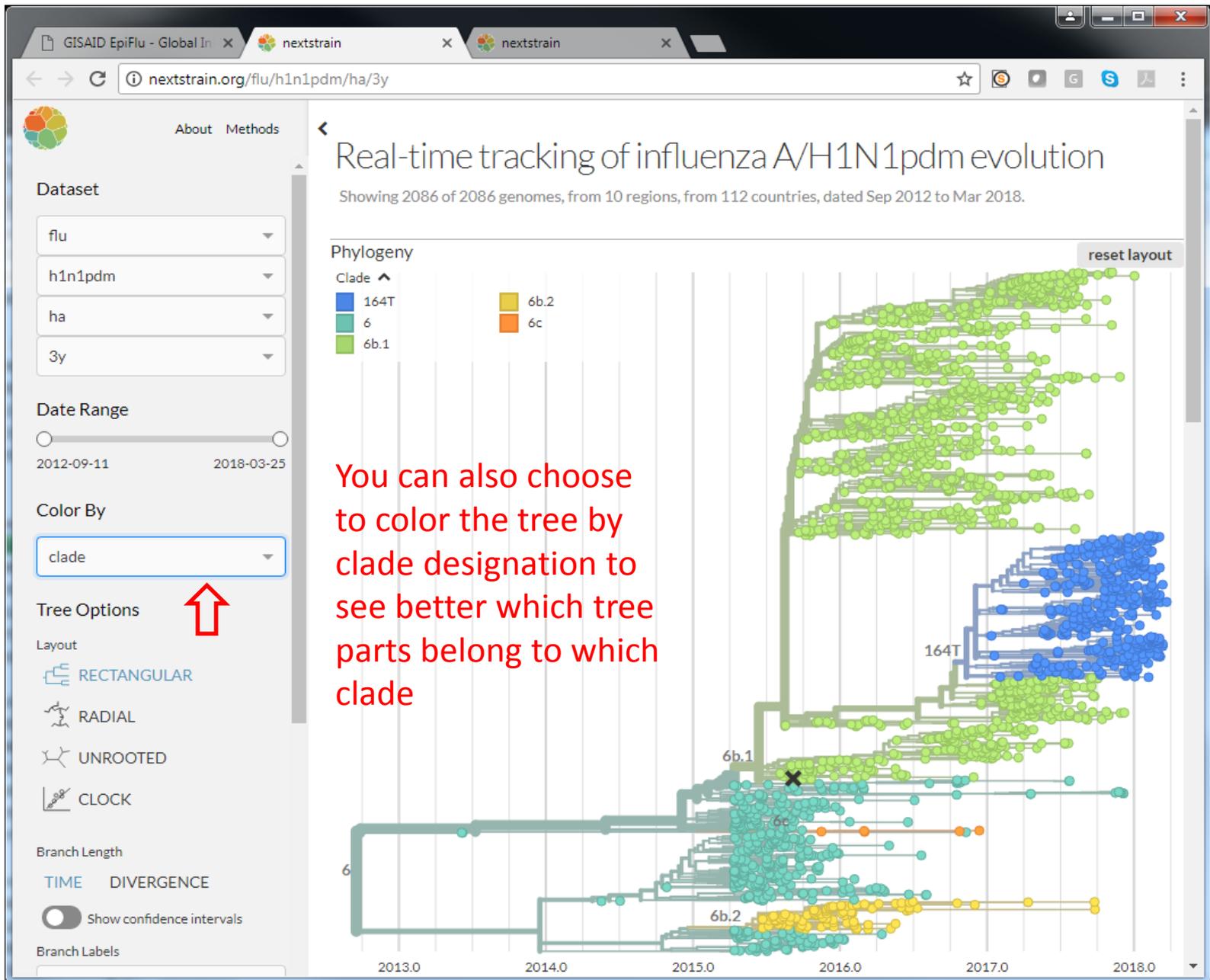
NEW: [Phylogenetic context for this mutation at NEXTFLU](#)



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Do the same now for the next mutation (S181T in FluSurver = HA1 164 in alternative numbering)





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FluSurver

Query	Best r
HA_A/India/1038/2017_295986	HA A/Michig find closest
HA_A/India/5869/2017_295888	HA A/Michig find closest
HA_A/India/6012/2017_295987	HA A/Michig find closest
HA_A/India/9929/2017_295884	HA A/Michig find closest

Warning: this reference selection includes segments to different reference

HA R240Q

Key to alternative position numbering:

240	FluSurver numbering (absolute as in 2009 H1N1 pandemic)
HA1 226	Classical H3N2 strain numbering
HA1 223	Classical H1N1 strain numbering

Chosen reference: HA_H1N1_Human_2015_Michigan45
 Position in reference: 240
 AA in reference: R
 AA in query: Q

Mutation HA R240Q already occurred 2485 times (99.84% of all samples with HA sequence) in 17 countries. The first strain with this mutation, collected in September 2015, was A/swine/Indians/A01944001/2015. The mutation most recently occurred in strain A/Utah/22/2017, collected in April 2017. ([see map](#))
[See detailed global statistics for this position](#)

A mutation at the position equivalent to HA 240 has been reported in the literature to be related to [host specificity shift and antigenic drift / escape mutant and other](#).

A combination of mutations including the position equivalent to HA 240 has been reported in the literature to be related to [host specificity shift](#).

As seen in resolved structures of proteins from related strains, the HA position equivalent to your mutation is involved in:

- [host cell receptor binding](#)
- [binding small ligand\(s\)](#)
- [antibody recognition sites](#)

[See all interactions for this position](#)

Passage bias info available:
 In subtype H1, Q at this position is preferred over R in MDCK cells with odds ratio of 37.14.

[PubMed search for this mutation \(including alternative numbering\)](#)

HA_240_IJSH_A_216 - Google Chrome

mendel.bii.a-star.edu.sg/METHODS/flusurver/beta/INTERACTIONS/HA/240/H...

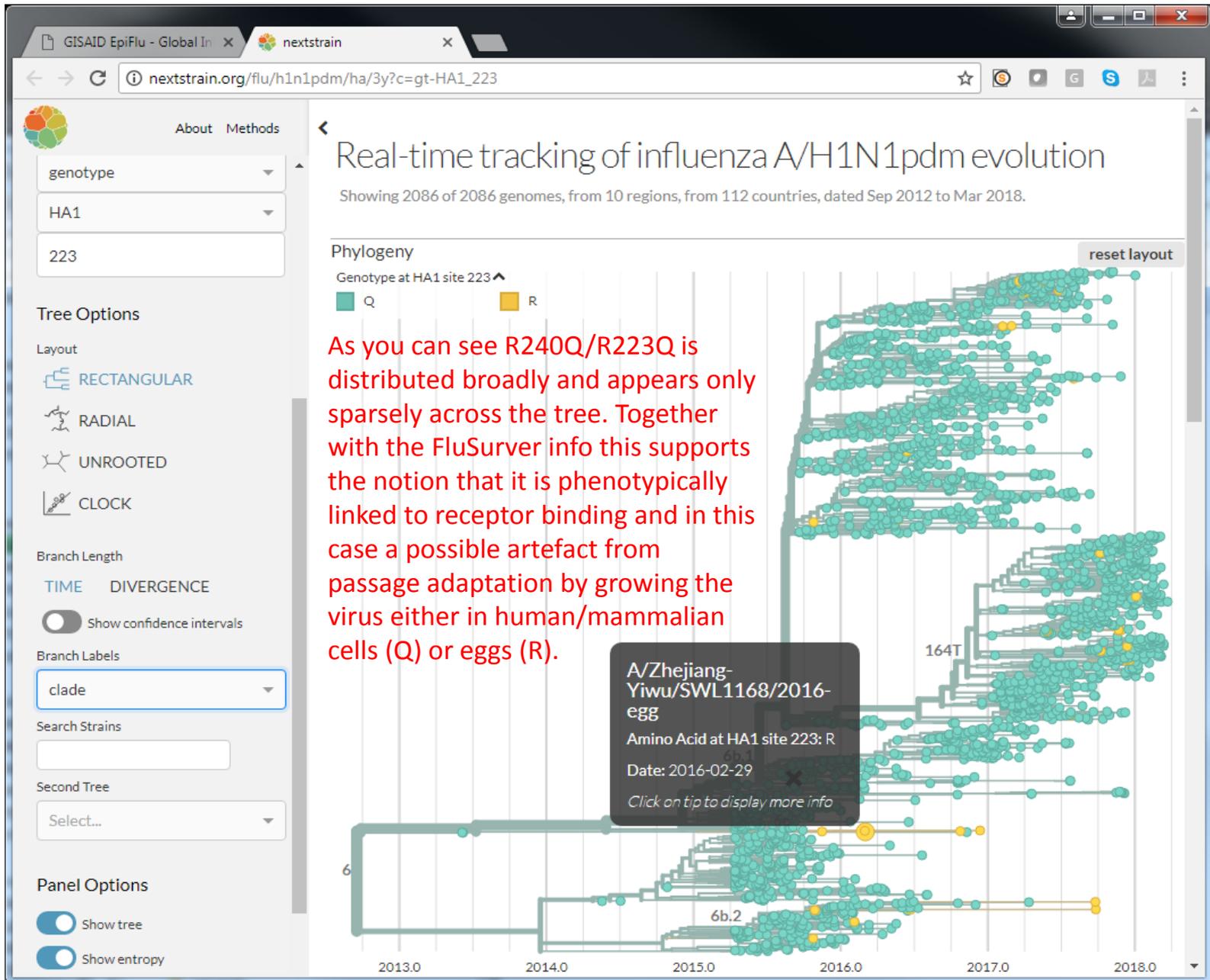
Mutation in receptor binding site

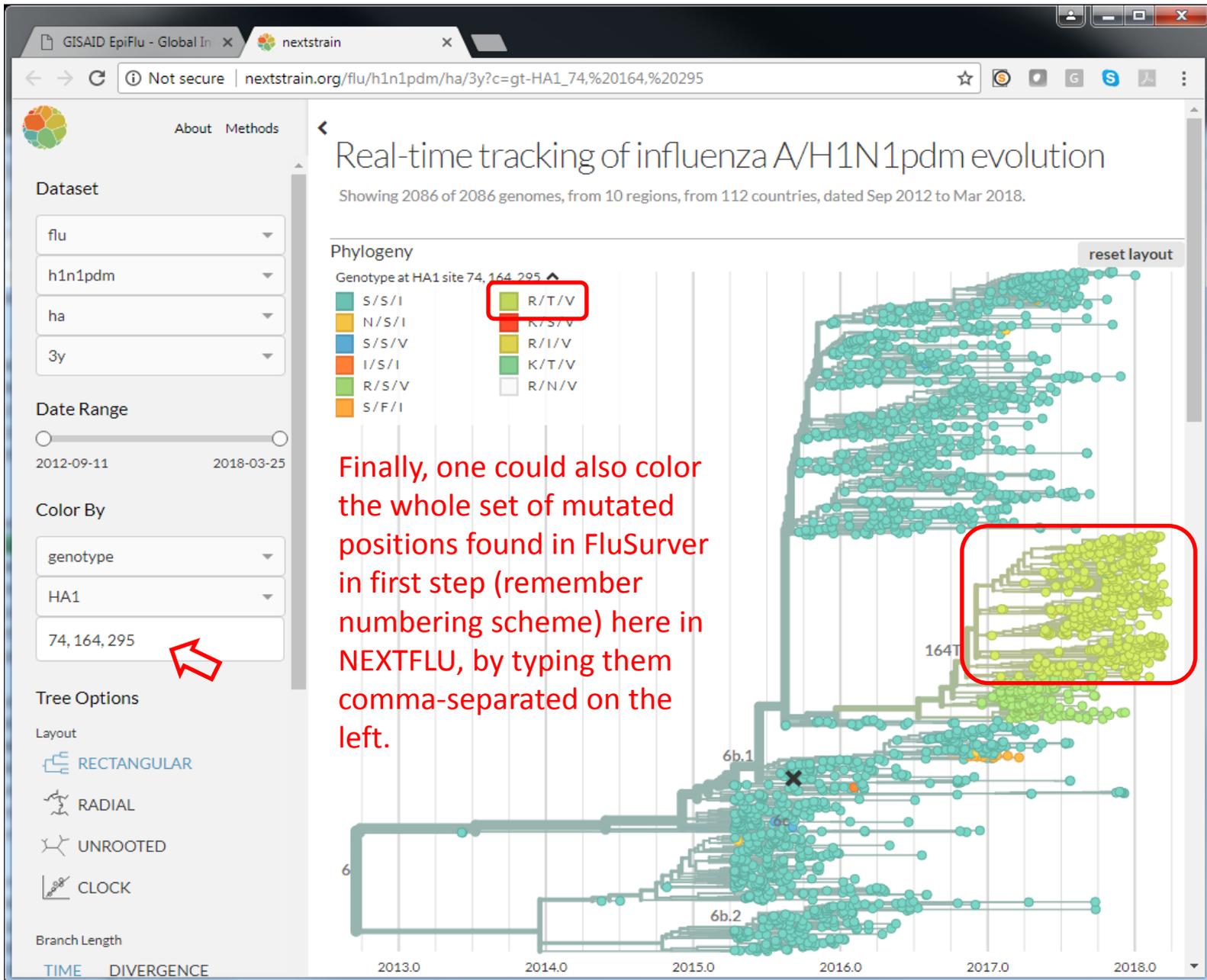
Spin ON Spin OFF Save IMAGE JSmol

Description: The mutation position (red atoms) corresponds to position 216 on viral chain A (yellow backbone) and is within 5 Å from host cell receptor GAL (pink atoms), host cell receptor SIA (pink atoms).

Legend: White backbone: other chains found in the structure
 See all 99 interactions for this position

Now let's look at R240Q/R223Q. Note the red warnings for host specificity and host receptor binding roles (click on them to learn more). There is also a passage bias note. Scroll down and click to see this mutation NEXTFLU.





Finally, one could also color the whole set of mutated positions found in FluSurver in first step (remember numbering scheme) here in NEXTFLU, by typing them comma-separated on the left.