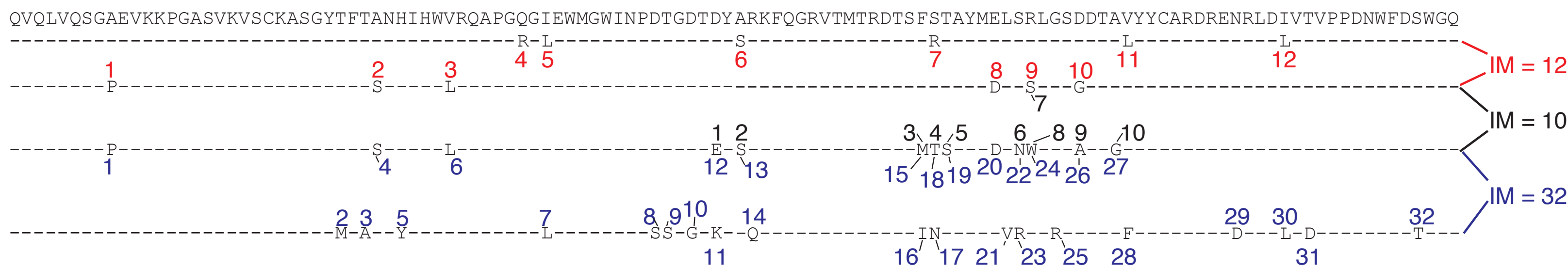
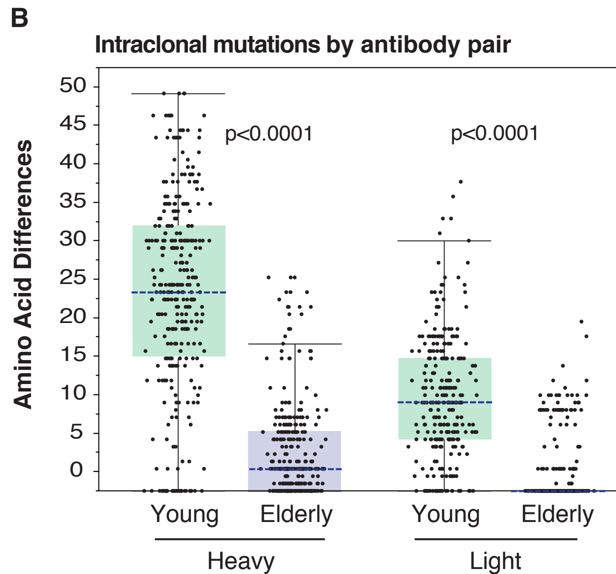
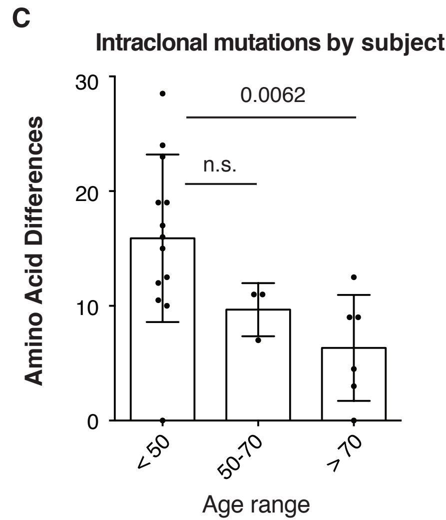
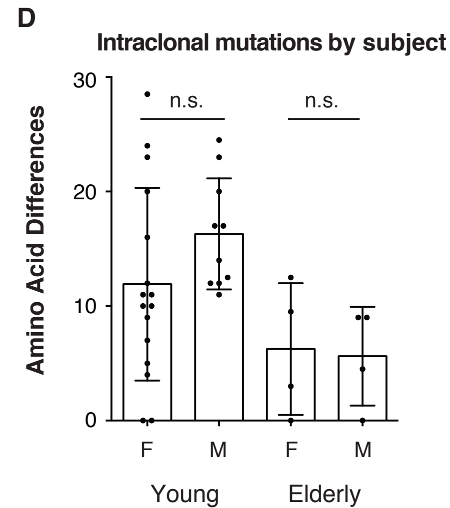
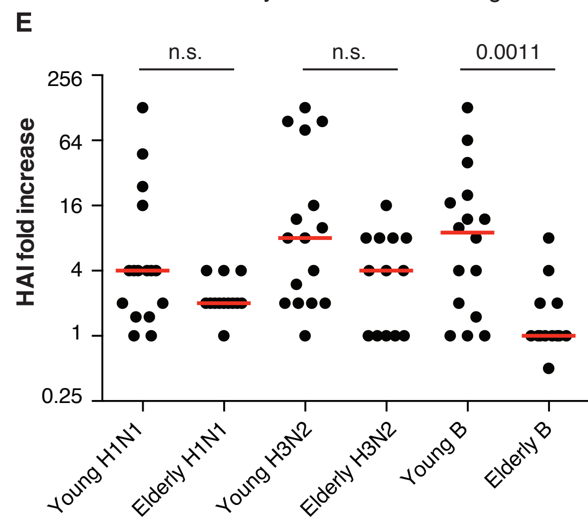
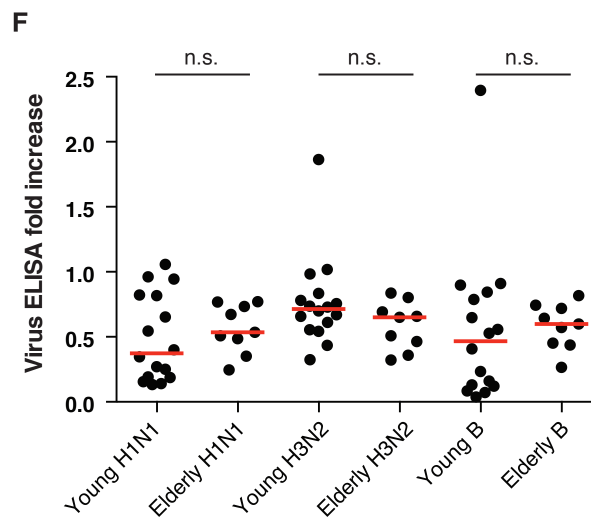

A
Consensus:
IM = Intraclonal Mutations
QVQLVQSGAEVKKPGASVKVSCKASGYTFTANHIHWVRQAPGQGIEWMGWINPDTGDTDYARKFQGRVTMTRDTSFSTAYMELSRLGSDDTAVYYCARDRENRLDIVTVPPDNWFDSWGQ
IM = 12
IM = 10
IM = 32

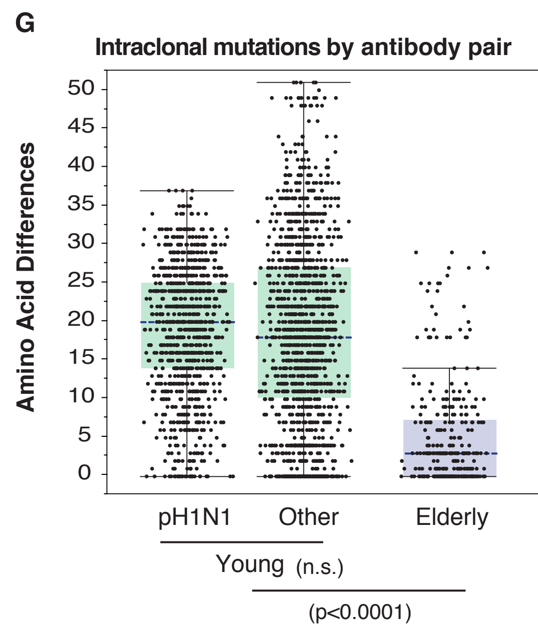

B
Intraclonal mutations by antibody pair
Amino Acid Differences
p<0.0001
p<0.0001
Young
Elderly
Young
Elderly
Heavy
Light


C
Intraclonal mutations by subject
Amino Acid Differences
0.0062
n.s.
<50
50-70
>70
Age range


D
Intraclonal mutations by subject
Amino Acid Differences
n.s.
n.s.
F
M
F
M
Young
Elderly


E
HAI fold increase
n.s.
n.s.
0.0011
Young H1N1
Elderly H1N1
Young H3N2
Elderly H3N2
Young B
Elderly B


F
Virus ELISA fold increase
n.s.
n.s.
n.s.
Young H1N1
Elderly H1N1
Young H3N2
Elderly H3N2
Young B
Elderly B


G
Intraclonal mutations by antibody pair
Amino Acid Differences
pH1N1
Other
Elderly
Young (n.s.)
(p<0.0001)

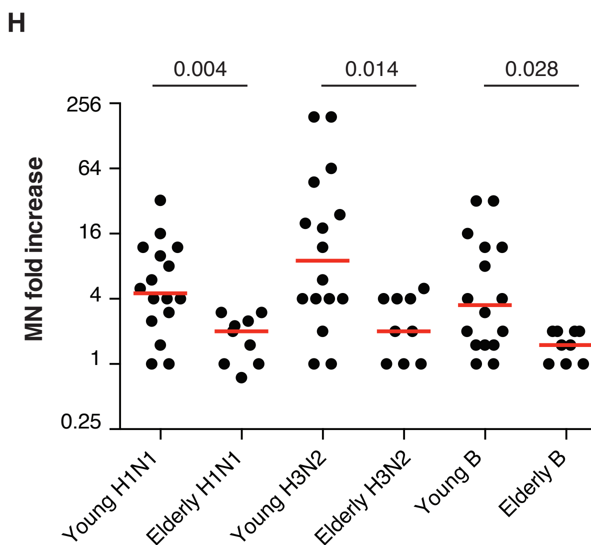
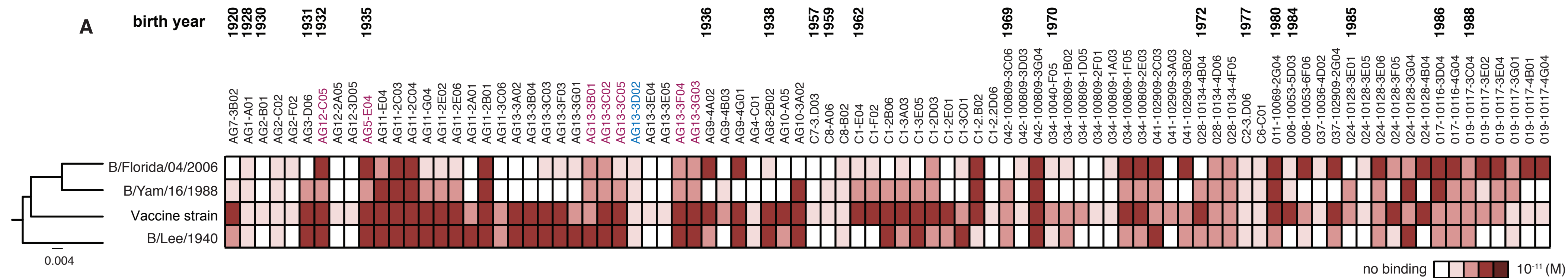
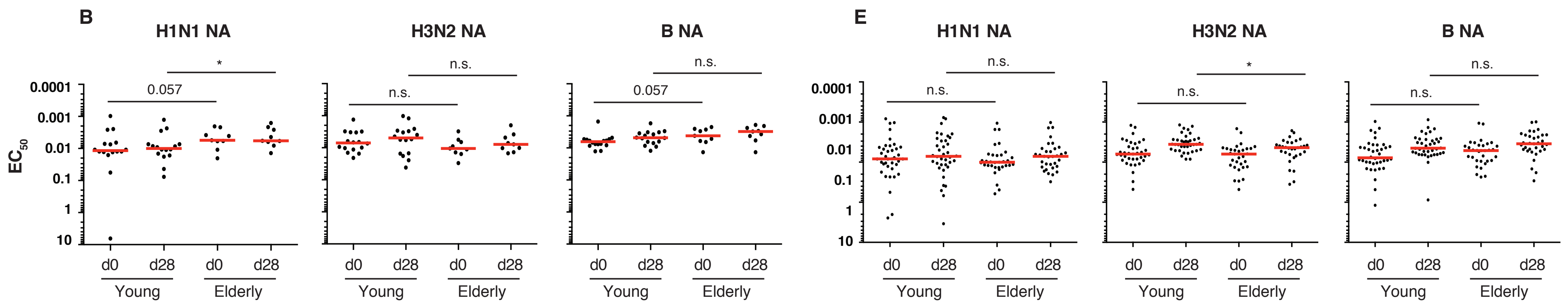
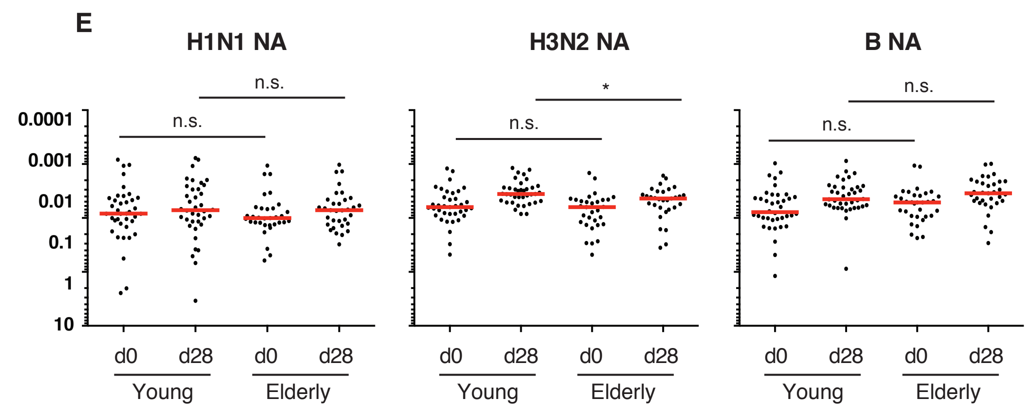

H
MN fold increase
0.004
0.014
0.028
Young H1N1
Elderly H1N1
Young H3N2
Elderly H3N2
Young B
Elderly B

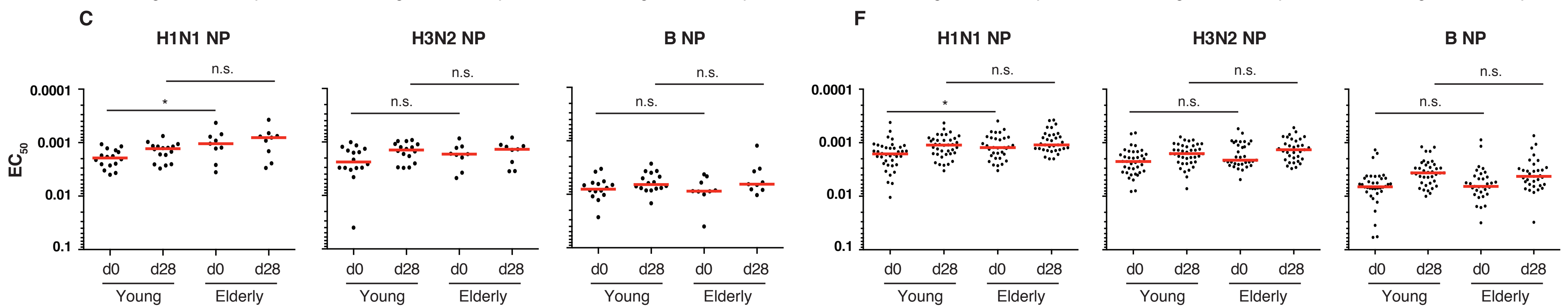
Figure S1. Reduced potency and capacity to target drifted epitopes by influenza-reactive antibodies from elderly individuals compared to young adults due to lack of de novo mutations, Related to Figures 1 and 3.

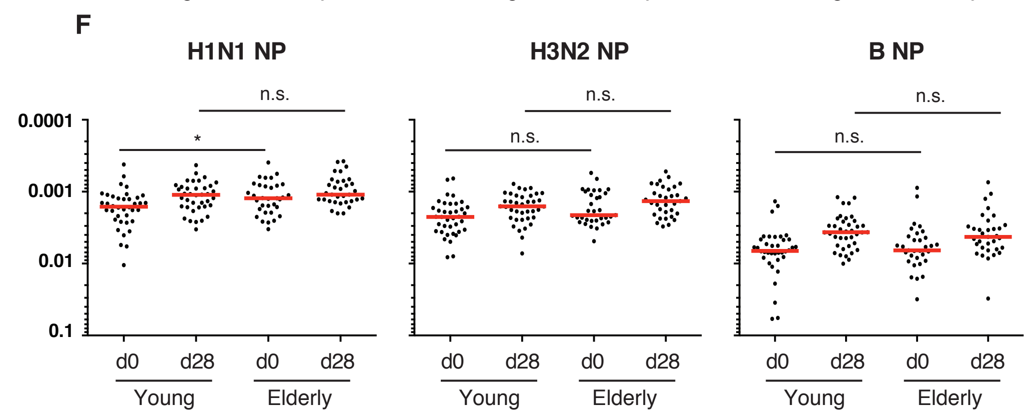
(A) Example of intraclonal diversity analysis between different VH sequences within a clonal lineage.
(B-D) Intraclonal mutations by antibody pair or by subject. Testing for statistical significance was performed using a Mann−Whitney U test. n.s. not significant.
(B) Variable distribution with quartiles is represented for each group and the median is represented as a blue dotted line. Amino-acid differences by antibody pair in each group (young adults n = 2465; aged n = 340) for the heavy and light chains. (C) Amino-acid differences by subject in three age groups: below 50, between 50 and 70 years old and over 70 years old. (D) Amino-acid differences by subject based on gender.
(E) Serum antibody HAI titers in the young cohort (n=16) compared to the elderly cohort (n=13) for H1N1 strains (A/California/07/09 and A/Brisbane/59/2007), H3N2 strains (A/Uruguay/716/2007 and A/Perth/16/2009) and B/Brisbane/60/2008 virus. Each dot represents the fold change increase between d0 and d28 in an individual donor. Medians are represented in red. Testing for statistical significance was performed using a Mann−Whitney U test.
(F) Serum antibody virus ELISA titers in the young cohort (n=16) compared to the elderly cohort (n=9) for H1N1 strains (A/California/07/09 and A/Brisbane/59/2007), H3N2 strains (A/Uruguay/716/2007 and A/Perth/16/2009) and B/Brisbane/60/2008 virus. Each dot represents the fold change increase between d0 and d28 in an individual donor. Medians are represented in red. Testing for statistical significance was performed using a Mann−Whitney U test.
(G) Intraclonal mutations by antibody pair represented as amino-acid differences for the heavy chain of the young cohort in response to seasonal influenza virus vaccination (TIV) compared to the pH1N1 2009 vaccination and compared to the elderly cohort in response to the seasonal TIV.
(H) Serum antibody MN titers in the young cohort (n=16) compared to the elderly cohort (n=9) for H1N1 strains (A/California/07/09 and A/Brisbane/59/2007), H3N2 strains (A/Uruguay/716/2007 and A/Perth/16/2009) and B/Brisbane/60/2008 virus. Each dot represents the fold change increase between d0 and d28 in an individual donor. Medians are represented in red. Testing for statistical significance was performed using a Mann−Whitney U test.

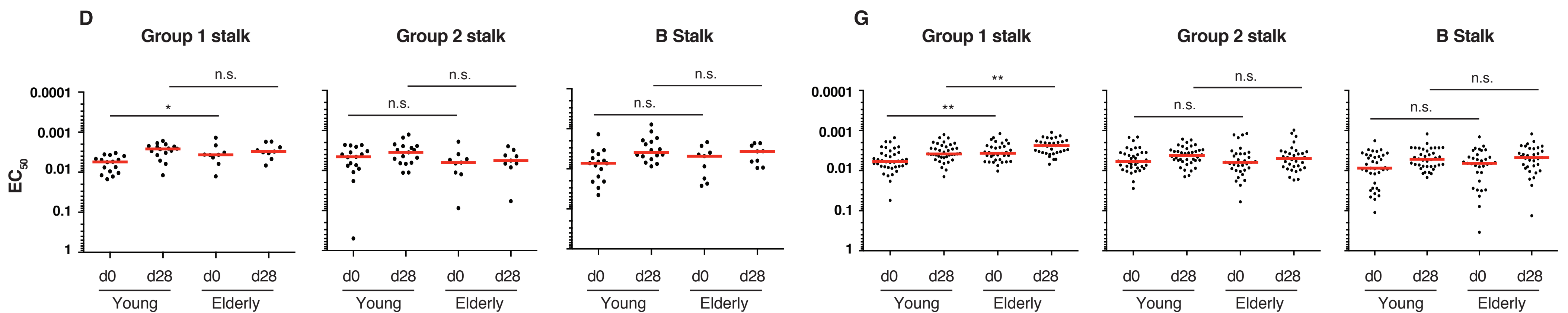

A
birth year
1920 1928 1930 1931 1932 1935 1936 1938 1957 1959 1962 1969 1970 1972 1977 1980 1984 1985 1986 1988
B/Florida/04/2006
B/Yam/16/1988
Vaccine strain
B/Lee/1940
0.004
no binding
10⁻¹¹ (M)


B
H1N1 NA
H3N2 NA
B NA
EC50
0.0001
0.001
0.01
0.1
1
10
0.057
*
n.s.
d0 d28 d0 d28
Young Elderly


E
H1N1 NA
H3N2 NA
B NA
n.s.
*
d0 d28 d0 d28
Young Elderly


C
H1N1 NP
H3N2 NP
B NP
EC50
0.0001
0.001
0.01
0.1
*
n.s.
d0 d28 d0 d28
Young Elderly


F
H1N1 NP
H3N2 NP
B NP
*
n.s.
d0 d28 d0 d28
Young Elderly


D
Group 1 stalk
Group 2 stalk
B Stalk
EC50
0.0001
0.001
0.01
0.1
1
*
n.s.
d0 d28 d0 d28
Young Elderly

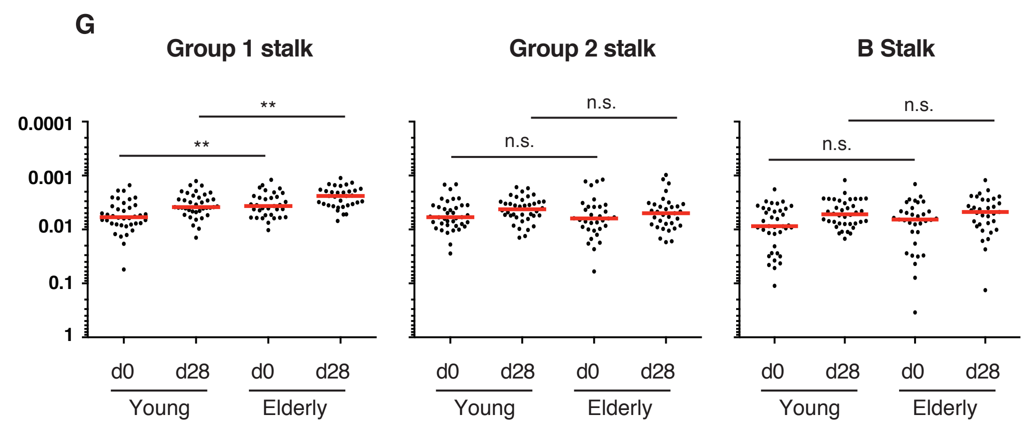
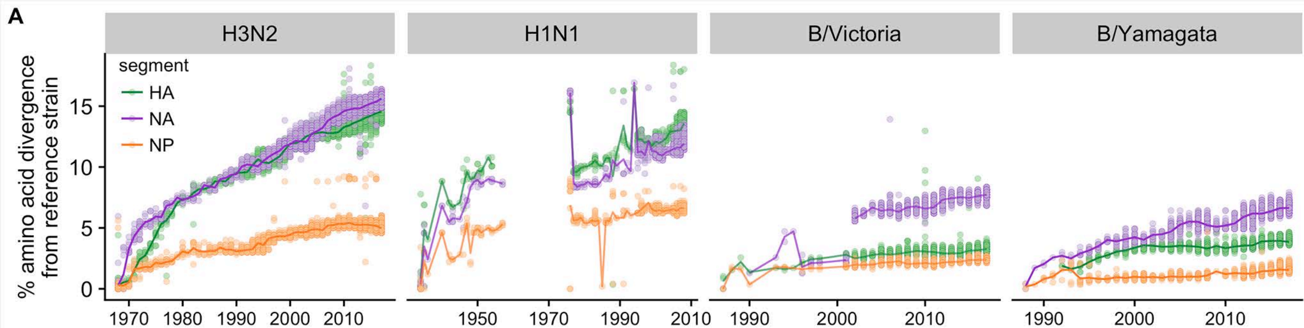

G
Group 1 stalk
Group 2 stalk
B Stalk
**
n.s.
d0 d28 d0 d28
Young Elderly

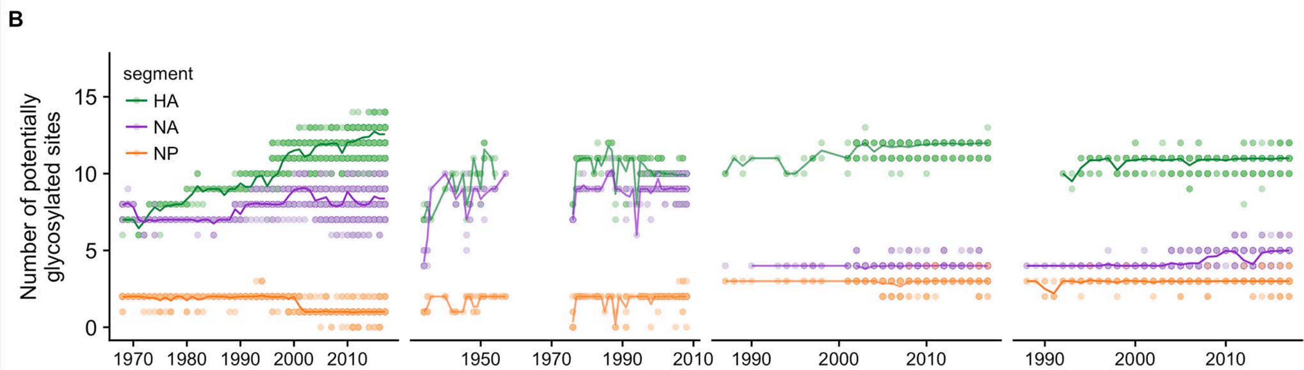
Figure S2. Influenza-reactive antibodies from elderly individuals target conserved epitopes on HA and on other viral proteins, Related to Figure 3.

(A) Cross-reactivity to past influenza B strain rHA proteins was tested for both cohorts by ELISA. The assays were performed in duplicate three times for each antibody. ELISA binding affinities represented by K_D (M) were plotted as a heatmap. The different HAs were clustered by amino acid sequence phylogeny, and multiple alignments were performed using the Clustal Omega algorithm. A rooted tree was constructed using the neighbor-joining method and was visualized using FigTree v1.4.0 software. The antibody in blue cross-reacted to influenza A and B strains. The antibodies in magenta are the MN^+ non-competing but cross-reactive antibodies. Vaccine strains are B/Brisbane/60/2008 and B/Malaysia/2506/2004.

(B-D**)** Serum antibody titers pre (d0) and post (d28) vaccination to the NA, NP and HA-stalk proteins in the young cohort (n=16) compared to the elderly cohort (n=9). Each dot represents a subject's EC_{50}. The assays were performed in duplicate twice. Medians are represented in red. Testing for statistical significance was performed using a Mann−Whitney U test. (B) Serum antibody titers to H1N1 NA proteins (A/California/04/09 or A/Brisbane/59/2007), H3N2 A/Wisconsin/ 57/2005 and B/Brisbane/60/2008 NA proteins. (C) Serum antibody titers to H1N1 NP A/Puerto Rico/8/1934, H3N2 NP A/Aichi/2/1968 and B/Florida/4/2006 NP proteins. (D) Serum antibody titers to group 1 stalk HA (chimeric H6/1 with A/Puerto Rico/8/1934 stalk or cH6/1 with A/California/04/09 stalk), group 2 stalk HA (cH5/3 with A/Perth/16/2009 stalk) and B stalk (cH8/B with B/Florida/4/2006 stalk).

(E-G) Serum antibody titers pre (d0) and post (d28) vaccination to the NA, NP and HA-stalk proteins in additional young (n=38) and elderly (n=33) cohorts. Each dot represents a subject's EC_{50}. The assays were performed in duplicate twice. Medians are represented in red. Testing for statistical significance was performed using a Mann−Whitney U test. (E) Serum antibody titers to H1N1 A/California/04/09, H3N2 A/Wisconsin/57/2005 or A/Hong-Kong/485197/2014 and B/Brisbane/60/2008 NA proteins. (F) Serum antibody titers to H1N1 NP A/Puerto Rico/8/1934, H3N2 NP A/Aichi/2/1968 and B/Florida/4/2006 NP proteins. (G) Serum antibody titers to group 1 stalk HA (cH6/1 with A/California/04/09 stalk), group 2 stalk HA (cH5/3 with A/Perth/16/2009 stalk) and B stalk (cH8/B with B/Florida/4/2006 stalk).





C

domain
HA head
HA stem
NA head
NA non-head
NA active sites

% amino acid divergence from reference strain

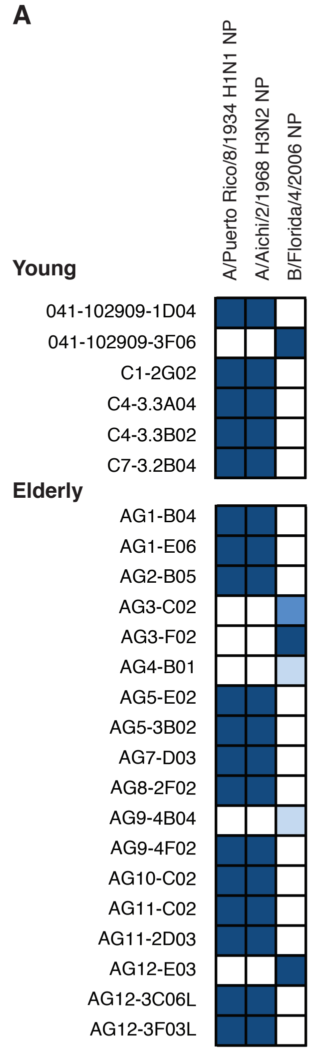
0
10
20
30

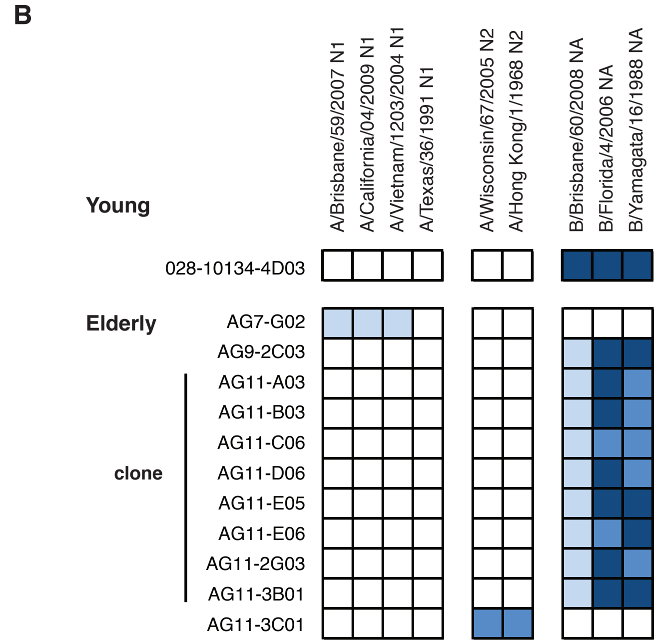
1970
1980
1990
2000
2010
1950
1970
1990
2010
1990
2000
2010
1990
2000
2010

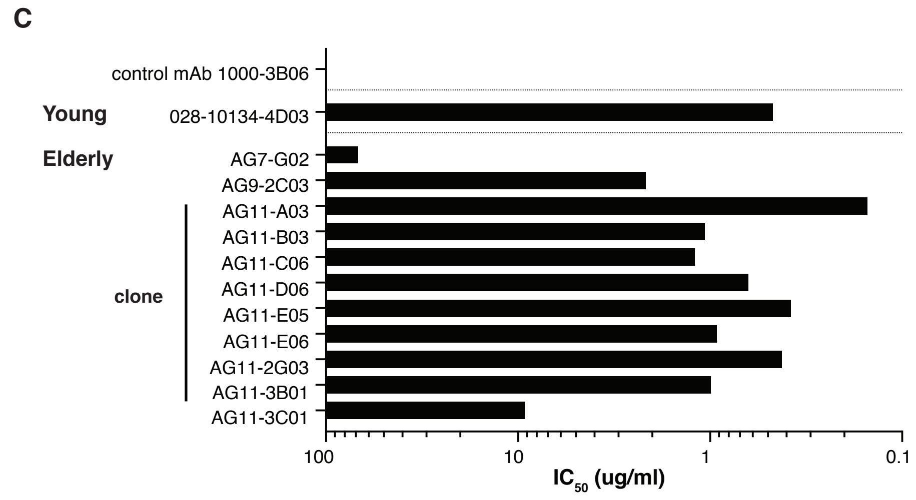
Year

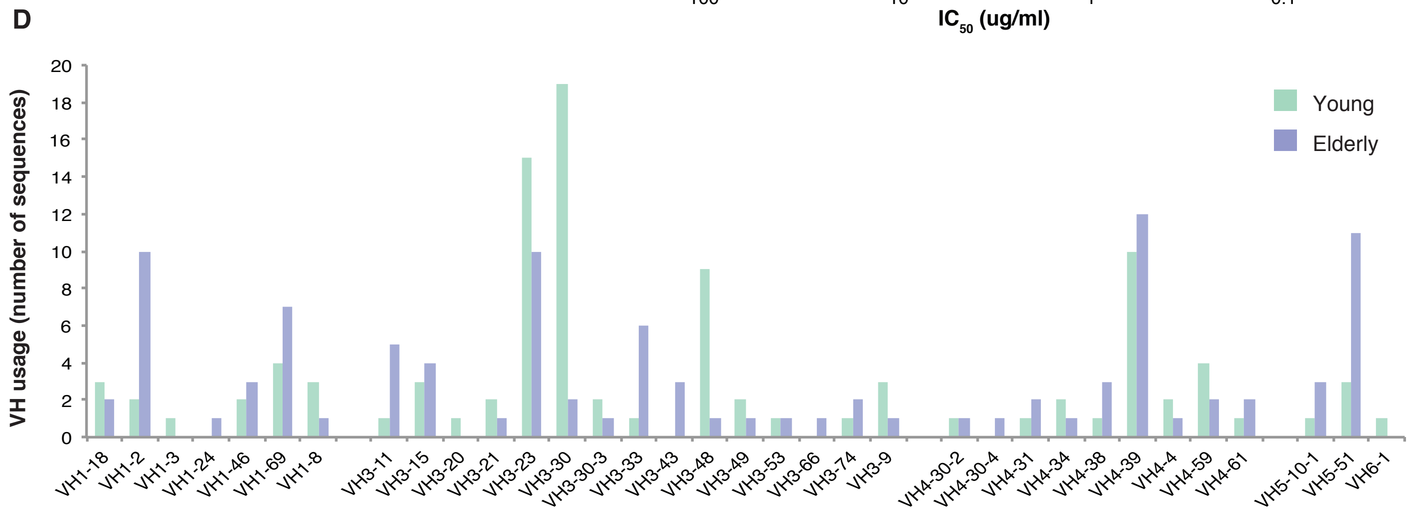
Figure S3. Evolutionary changes in the hemagglutinin (HA), neuraminidase (NA) and nucleoprotein (NP) segments of influenza A and B viruses, Related to Figures 3 and 4.

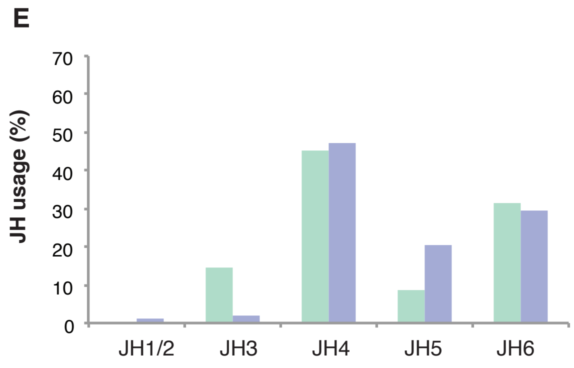
(A) Percent amino acid sequence divergence relative to reference strains A/Hong Kong/01/1968 (H3N2), A/Puerto Rico/8-1/1934 (H1N1), B/Victoria/02/87 (B/Victoria) and B/Yamagata/16/88 (B/Yamagata). (B) Number of potentially N-linked glycosylated sites identified from N-X-[ST]-X motifs, where X is any amino acid except proline. (C) Percent amino acid divergence over time for different domains of HA and NA. Analyses were based on complete sequences for the protein coding regions of each segment isolated until 2017 and obtained from the NCBI Influenza Virus Database. Lines indicate per-year means. The discontinuity in the B/Victoria NA corresponds to acquisition of a Yamagata-lineage NA by Victoria viruses through reassortment in the early 2000s (Langat et al., 2017).


A
A/Puerto Rico/8/1934 H1N1 NP
A/Aichi/2/1968 H3N2 NP
B/Florida/4/2006 NP
Young
041-102909-1D04
041-102909-3F06
C1-2G02
C4-3.3A04
C4-3.3B02
C7-3.2B04
Elderly
AG1-B04
AG1-E06
AG2-B05
AG3-C02
AG3-F02
AG4-B01
AG5-E02
AG5-3B02
AG7-D03
AG8-2F02
AG9-4B04
AG9-4F02
AG10-C02
AG11-C02
AG11-2D03
AG12-E03
AG12-3C06L
AG12-3F03L


B
A/Brisbane/59/2007 N1
A/California/04/2009 N1
A/Vietnam/1203/2004 N1
A/Texas/36/1991 N1
A/Wisconsin/67/2005 N2
A/Hong Kong/1/1968 N2
B/Brisbane/60/2008 NA
B/Florida/4/2006 NA
B/Yamagata/16/1988 NA
Young
028-10134-4D03
Elderly
AG7-G02
AG9-2C03
clone
AG11-A03
AG11-B03
AG11-C06
AG11-D06
AG11-E05
AG11-E06
AG11-2G03
AG11-3B01
AG11-3C01
< 0.37μg/ml
10-0.37μg/ml
10μg/ml


C
control mAb 1000-3B06
Young
028-10134-4D03
Elderly
AG7-G02
AG9-2C03
clone
AG11-A03
AG11-B03
AG11-C06
AG11-D06
AG11-E05
AG11-E06
AG11-2G03
AG11-3B01
AG11-3C01
100
10
1
0.1
IC50 (ug/ml)


D
VH usage (number of sequences)
0
2
4
6
8
10
12
14
16
18
20
Young
Elderly
VH1-18
VH1-2
VH1-3
VH1-24
VH1-46
VH1-69
VH1-8
VH3-11
VH3-15
VH3-20
VH3-21
VH3-23
VH3-30
VH3-30-3
VH3-33
VH3-43
VH3-48
VH3-49
VH3-53
VH3-66
VH3-74
VH3-9
VH4-30-2
VH4-30-4
VH4-31
VH4-34
VH4-38
VH4-39
VH4-4
VH4-59
VH4-61
VH5-10-1
VH5-51
VH6-1


E
JH usage (%)
0
10
20
30
40
50
60
70
JH1/2
JH3
JH4
JH5
JH6

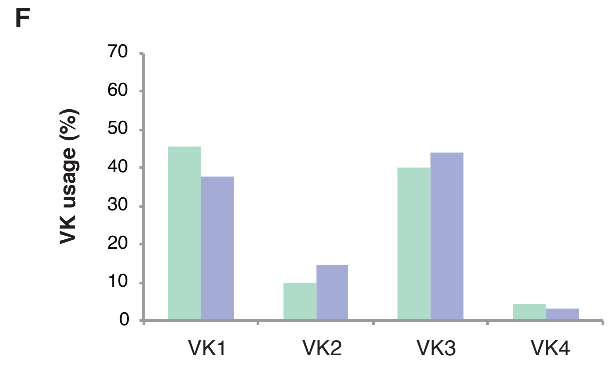

F
VK usage (%)
0
10
20
30
40
50
60
70
VK1
VK2
VK3
VK4

Figure S4. Influenza-reactive antibodies from elderly individuals target conserved epitopes on NA and NP, Related to Figure 3.

(A) Virus-positive non-HA reactive antibodies were tested for NP reactivity by ELISA. Strains used were A/Puerto Rico/8/1934 for H1N1, A/Aichi/2/1968 for H3N2, and B/Florida/4/2006. Shown is a heatmap of minimum positive concentration (in µg/ml) for all NP-reactive antibodies. The intensity of blue correlates with the binding activity (lower antibody concentration to bind to NP proteins). White indicates no binding. The assays were performed in duplicate three times for each antibody.

(B) Virus-positive non-HA reactive antibodies were tested for NA reactivity by ELISA. A panel of N1, N2, and B NAs was used. Shown is a heatmap of minimum positive concentration (in µg/ml) for all NA-reactive antibodies. The intensity of blue correlates with the binding activity (lower antibody concentration to bind to NA proteins). White indicates no binding. The assays were performed in duplicate three times for each antibody.

(C) NA-reactive antibodies were tested for NA enzymatic activity using a NA-*Star* assay. A/Brisbane/59/2007 H1N1, A/Wisconsin/67/2005, and B/Florida/04/2006 viruses were tested. Shown are the IC_{50} (in µg/ml) for all NA-reactive antibodies. The IC_{50} were defined as the concentration at which 50% of the NA activity was inhibited. The control antibody (1000-3B06) was used (NA-reactive antibody that does not inhibit the enzymatic active site in the NA-*Star* assay). The experiment was done in duplicate three times.

(D-F) Molecular characteristics of the immunoglobulin genes coding for all influenza-reactive antibodies (young cohort, n=102; elderly cohort, n=102). Ig genes coding for each antibody was analyzed using V-Genes and IMGT to determine the (D) VH usage (E) JH usage and (F) the VK repertoire. In (D) are shown the total number of sequences for a particular VH gene. In (E) and (F) are shown the percentages of mAbs in each family.

Table S1. Information on the subject's age, gender, year of vaccination, and type of vaccine received, Related to Figure 1.

	Subject ID	Age	Gender	Vaccine	Type of vaccine
Young subjects	007	23	F	2010-2011	Fluvirin
	008	26	F	2010-2011	Fluvirin
	009	25	F	2010-2011	Fluvirin
	011	30	M	2010-2011	Fluvirin
	012	23	M	2010-2011	Fluvirin
	013	24	F	2010-2011	Fluvirin
	017	24	M	2010-2011	Fluvirin
	019	22	F	2010-2011	Fluvirin
	024	25	M	2010-2011	Fluvirin
	028	38	M	2010-2011	Fluvirin
	030	32	F	2010-2011	Fluvirin
	042	40	M	2009-2010	Fluzone
	037	26	F	2010-2011	Fluvirin
	034	39	F	2009-2010	Fluzone
	041	23	M	2009-2010	Fluzone
	045	24	M	pand. 2009	Monovalent vaccine
	047	23	M	pand. 2009	Monovalent vaccine
	051	43	M	pand. 2009	Monovalent vaccine
	SFV005	60	F	pand. 2009	Monovalent vaccine
	SFV009	31	F	pand. 2009	Monovalent vaccine
	SFV015	48	F	pand. 2009	Monovalent vaccine
	SFV018	58	F	pand. 2009	Monovalent vaccine
	SFV019	48	F	pand. 2009	Monovalent vaccine
	SFV020	64	F	pand. 2009	Monovalent vaccine
	C1	44	M	Y1 2006-2007	Fluvirin
				Y2 2007-2008	Fluvirin
				Y3 2008-2009	Fluzone
	C2	30	F	Y2 2007-2008	Fluvirin
				Y3 2008-2009	Fluzone
	C3	29	F	Y2 2007-2008	Fluvirin
				Y3 2008-2009	Fluzone
	C4	43	F	Y2 2007-2008	Fluvirin
				Y3 2008-2009	Fluzone
	C5	39	F	Y1 2006-2007	Fluvirin
	C6	29	F	Y1 2006-2007	Fluvirin
				Y2 2007-2008	Fluvirin

	C7	51	M	Y3 2008-2009	Fluzone
	C8	47	F	Y1 2006-2007	Fluvirin
				Y2 2007-2008	Fluvirin
Aged subjects	AG1	79	M	2007-2008	Fluzone
	AG2	77	M	2007-2008	Fluzone
	AG3	76	F	2007-2008	Fluzone
	AG4	71	F	2007-2008	Fluzone
	AG5	74	F	2009-2010	Fluzone
	AG6	78	M	2009-2010	Fluzone
	AG7	89	F	2009-2010	Fluzone
	AG8	71	F	2009-2010	Fluzone
	AG9	73	M	2009-2010	Fluzone
	AG10	71	M	2009-2010	Fluzone
	AG11	74	F	2009-2010	Fluzone
	AG12	77	M	2009-2010	Fluzone
	AG13	74	M	2009-2010	Fluzone

The pandemic 2009 H1N1 vaccine was the monovalent vaccine from Sanofi Pasteur.
The elderly individuals received the TIV in 2007-2008 or 2009-2010 while the younger adults received the TIV from 2006-2007 to 2010-2011. Matching strains between both cohorts were used for further experiments and were: A/Solomon Islands/3/2006 and A/Brisbane/59/2007 for H1N1, A/Wisconsin/67/2005 and A/Brisbane/10/2007 for H3N2 and B/Malaysia/2506/2004 and B/Brisbane/60/2008 for B strains.

Table S2. Numbers of influenza-reactive sequences, influenza-reactive clonal lineages and influenza-reactive monoclonal antibodies analyzed by subject, Related to Figure 1.

	Subject ID	Number of influenza-reactive VH sequences	Number of influenza-reactive clonal lineages	Number of influenza-reactive mAbs fully characterized
Young subjects	007	42	6	
	008	36	3	2
	009	62	7	
	011	26	4	1
	012	34	7	
	013	48	5	
	017	48	5	2
	019	30	2	6
	024	28	3	6
	028			4
	030	21	1	
	042	45	6	4
	037	6	1	2
	034	51	8	11
	041	25	2	8
	045	29	3	
	047	26	3	
	051	31	9	
	SFV005	4	1	
	SFV009	13	1	
	SFV015	49	4	12*
	SFV018	5	1	
	SFV019	29	4	
	SFV020	11	2	
	C1 – Y1	52	8	16
	C1 – Y2			10
	C1 – Y3			2
	C2 – Y2			1

	C2 – Y3			4
	C3 – Y2			3
	C3 – Y3			1
	C4 – Y2	4	1	1
	C4 – Y3			2
	C5 – Y1	2	1	1
	C6 – Y1			2
	C6 – Y2			5
	C7 – Y3			4
	C8 – Y1			3
	C8 – Y2			1
Aged subjects	AG1			4
	AG2			9
	AG3			7
	AG4			2
	AG5	10	2	4
	AG6			2
	AG7	8	2	6
	AG8	10	2	8
	AG9	10	2	7
	AG10			5
	AG11	39	4	24
	AG12	14	1	11
	AG13	21	3	13

Out of the 32 young individuals recruited in our study, only 26 had clonally related sequences. Thus we performed the intraclonal mutation analysis with these 26 individuals.
* Monoclonal antibodies from the donor SFV015 were used as an example of adaptation in figure 2 but they are not part of the 102 monoclonal antibodies fully characterized for the young individuals as they were induced by the pandemic 2009 H1N1 vaccine.