Pre-existing subtype immunodominance shapes memory B cell recall response to influenza vaccination

Rodrigo B. Abreu[1], Greg A. Kirchenbaum[1*], Emily F. Clutter[1], Giuseppe A. Sautto[1], and Ted M. Ross[1,2#]

Journal Of Clinical Investigation

Supplementary Data

Supplementary Figure Captions:

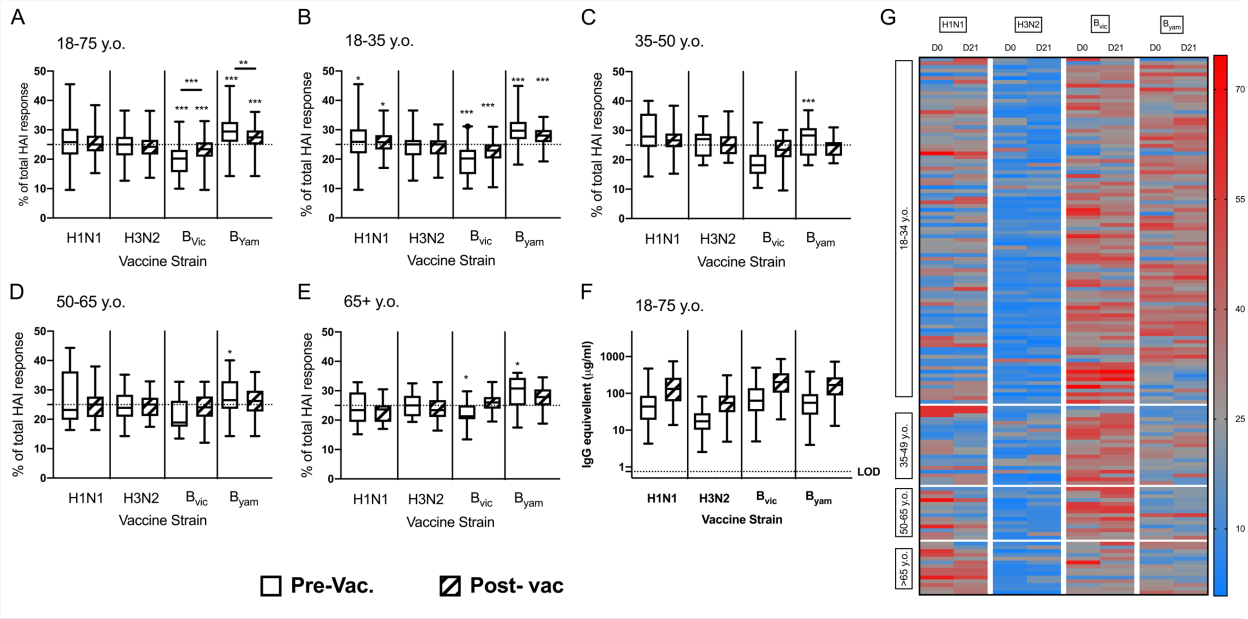

Fig S1: **Pre-existing subtype immune dominance persists after QIV vaccination.** A) Percentage HAI activity for each vaccine strain from the cumulative HAI response to QIV in healthy volunteers (18 to 75 y.o.) prior to and 21-28 days following vaccination. Horizontal dashed line represents the theoretical 25% response for each of the 4 vaccine strains in the absence of antigenic immunodominance. B-E) Percentage HAI activity as in A stratified by age group; B) 18-35 y.o. young adults, C) 35-50 y.o. adults, D) 50-65 y.o. middle age and E) 65+ y.o. elderly subjects. F) HA-specific IgG levels in the serum of healthy volunteers (18 to 75 years old) prior to and 21-28 days following QIV vaccination measured by quantitative ELISA against the four vaccine strains as described the M&M section. G) Subject heatmap showing the % of HA-binding to each vaccine component prior- and post-vaccination.

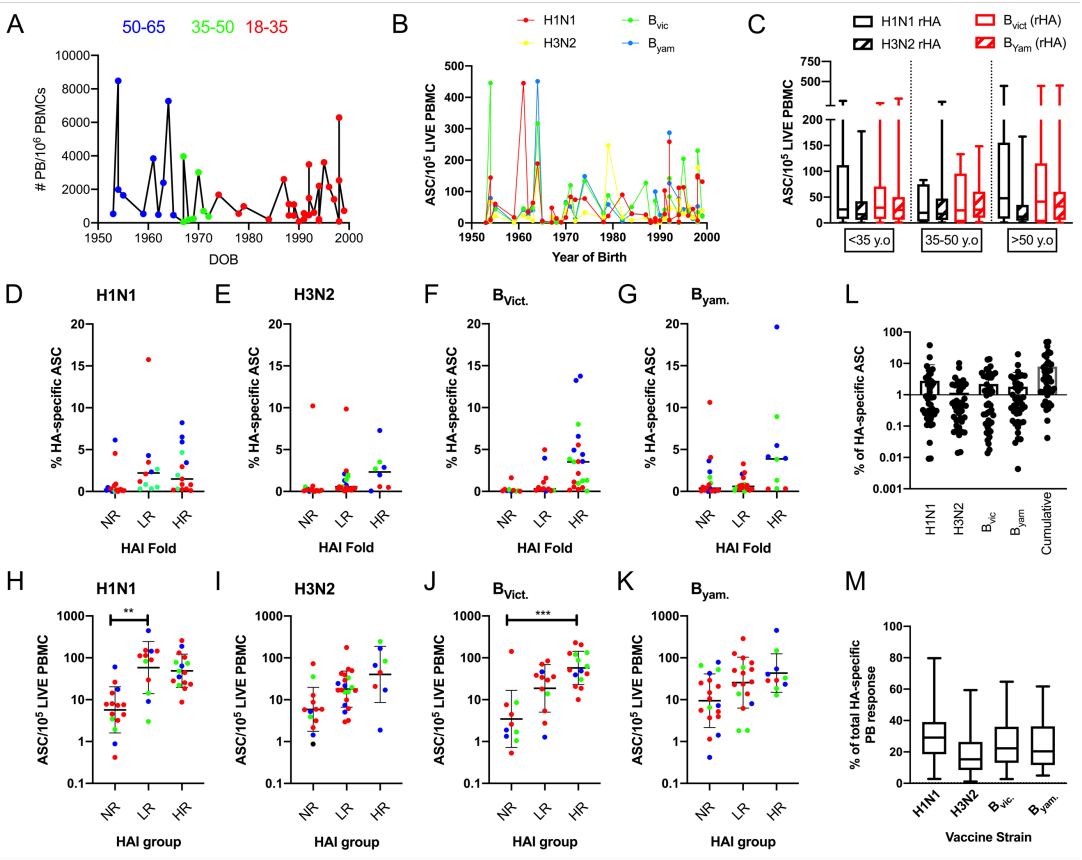

Fig S2: **Percentage of HA-specific plasmablast (PB) correlates with serological HAI activity.** A) Number of PB in the peripheral blood of 40 subjects of varying age 7 days after QIV vaccination. B-C) Number of rHA-specific antibody secreting cells (ASC), against each vaccine strain, in the peripheral blood of 40 healthy subjects of varying age, 7-9 days after vaccination measured by ELISpot. D-G) Percentage of rHA-specific ASC, against each vaccine strain, in the peripheral blood of 40 healthy subjects with no-(NR), low-(LR), or High-Response to the vaccine measured by HAI. H-K) Number of rHA-specific ASC, against each vaccine strain, in the peripheral blood of 40 healthy subjects with NR, LR or HR to the vaccine measured by HAI. L) Percentage of rHA-specific ASC against each vaccine component and cumulative HA-specific ASC, relative to total IgG ASC, in the peripheral blood of 40 healthy subjects of varying age, 7-9 days after vaccination. E) Percentage of rHA-specific ASC against each vaccine component, relative to the cumulative HA-specific response in the peripheral blood of 40 healthy subjects of varying age, 7-9 days after vaccination.

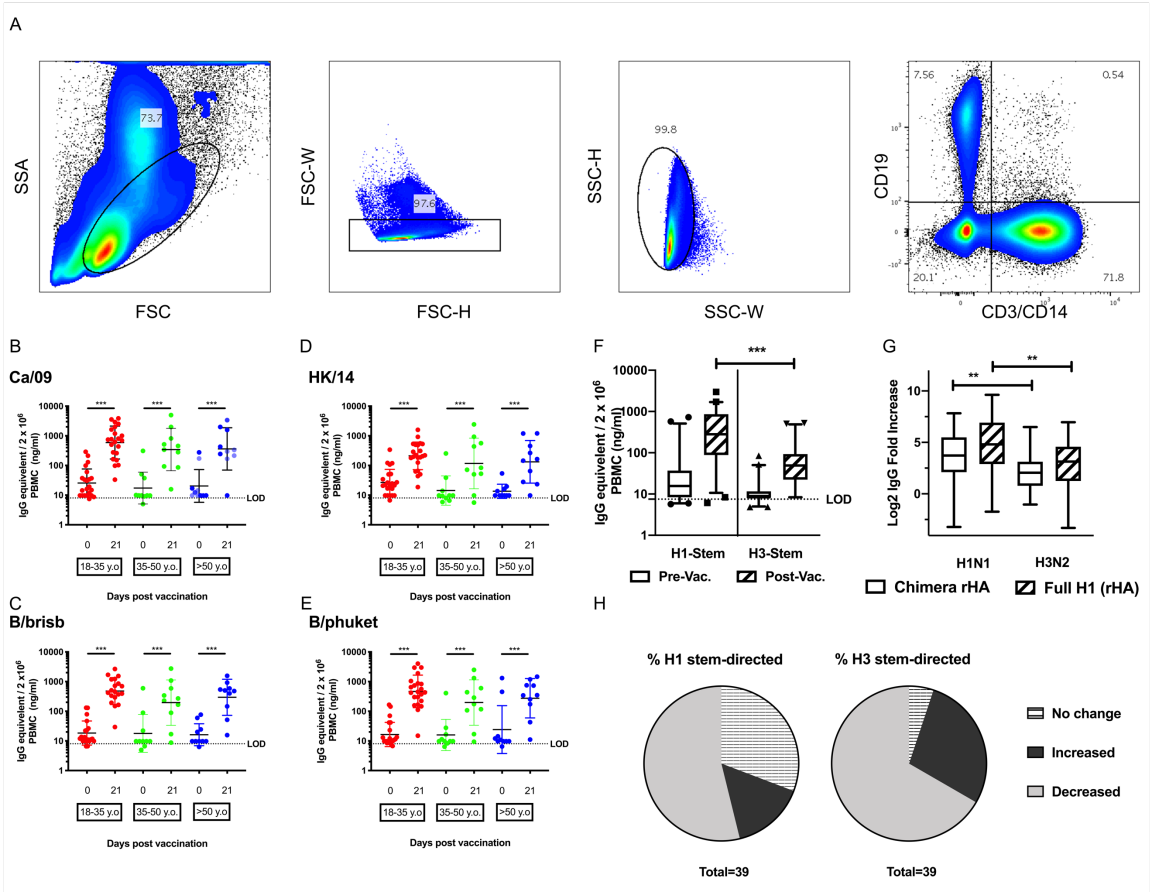

Fig S3: **Immunodominant H1N1 B_{mem} response to stem following QIV vaccination.** A) Levels of HA-specific memory B cell (B_{mem})-derived antibody against each vaccine component, in 40 subjects of varying age, prior to and 21-28 days following vaccination, after *in vitro* differentiation as described in the M&M section. B) Fold change following vaccination, in levels of HA-specific B_{mem}-derived IgG antibodies against each vaccine component, measured as in A in 40 donors of varying age. C) Levels of cH6/H1-reactive and cH7/H3-reactive B_{mem}-derived IgG, prior to and 21-28 days following vaccination, following *in vitro* differentiation as described in the M&M section. D) Fold change following vaccination, in HA_{stem}-directed and full HA-binding B_{mem}-derived IgG against H1N1 and H3N2 vaccine strains, in 40 subjects, measured as in C. E) Proportion of subjects with a significant increase or decrease in the percentage of stem-directed B_{mem}-derived IgG against H1N1 and H3N2 vaccine strains, following vaccination. PBMC were differentiated for 7-9 dyas as described in M&M section

Table S1: Pearson correlations between assays and timepoints

D0/D21 Intra-assay	HAI		IgG		D0/D21 Immunodominance	IgG		HAI	
	r	*p*	r	*p*		r	*p*	r	*p*
H1N1	0.485	<0.0001	0.536	<0.0001	**H1N1**	0.642	<0.0001	0.488	<0.0001
H3N2	0.422	<0.0001	0.452	<0.0001	**H3N2**	0.349	0.001	0.4	<0.0001
Bvic	0.591	<0.0001	0.559	<0.0001	**Bvic**	0.284	0.011	0.457	<0.0001
Byam	0.467	<0.0001	0.387	0.0004	**Byam**	0.139	0.219	0.586	<0.0001

IgG(µg/ml)/ HAI Titer	D0		D21		IgG/HAI Immunodominance	D0		D21	
	r	*p*	r	*p*		r	*p*	r	*p*
H1N1	0.519	<0.0001	0.592	<0.0001	**H1N1**	0.247	0.027	0.232	0.038
H3N2	0.415	<0.0001	0.625	<0.0001	**H3N2**	0.517	<0.0001	0.49	<0.0001
Bvic	0.287	<0.0001	0.439	<0.0001	**Bvic**	0.43	<0.0001	0.327	0.0031
Byam	0.366	<0.0001	0.433	<0.0001	**Byam**	0.429	<0.0001	0.352	0.001

IgG_Bmem/ IgG_ELISA	D0		D21		Bmem/ ELISA Immunodominance	D0		D21	
	r	*p*	r	*p*		r	*p*	r	*p*
H1N1	-0.011	0.945	0.385	0.012	**H1N1**	0.301	0.057	0.374	0.014
H3N2	-0.071	0.656	0.427	0.005	**H3N2**	-0.063	0.688	0.232	0.14
Bvic	0.212	0.183	0.214	0.197	**Bvic**	0.216	0.168	0.404	0.011
Byam	-0.094	0.558	0.296	0.06	**Byam**	0.026	0.868	0.486	0.001

IgG_Bmem/ HAI Titer	D0		D21		Bmem/HAI Immunodominance	D0		D21	
	r	*p*	r	*p*		r	*p*	r	*p*
H1N1	0.348	0.025	0.29	0.065	**H1N1**	0.542	<0.001	0.521	<0.001
H3N2	0.086	0.593	0.303	0.053	**H3N2**	0.054	0.736	0.159	0.315
Bvic	0.103	0.522	0.209	0.208	**Bvic**	0.077	0.626	0.37	0.02
Byam	0.105	0.514	0.17	0.29	**Byam**	0.13	0.411	0.545	<0.001

51

Tables S2: Antibodies and dilutions

Antibody*	Cat#	Dilution
IgD BV421	348226	1/200
CD20 BV650	302334	1/50
CD3 FITC	300306	1/200
CD14 FITC	301804	1/200
CD38 Pe-Dazzel	303538	1/50
CD27 Pe-Cy7	124216	1/100
CD19 APC-Fire	302258	1/100

* All antibodies Purchuased from Biolegend

52