

A/USSR/90/1977 APLQLGKNCIAGWILGNPECESLFSKKSWSYIAETPNSENGTCYPGYFAD 101
A/Kawasaki/6/1986 APLQLGNCS IAGWILGNPECESLFSKKSWSYIAETPNSENGTCYPGYFAD
A/Massachusetts/1/1990 APLQLGNCS IAGWILGNPECESLFSKESWSYIAETPNSENGTCYPGYFAD
A/Wellington/47/1992 APLQLGNCS IAGWILGNPECESLFSKESWSYIAETPDPENGTCYPGYFAD
A/Florida/2/1993 APLQLGNCSVAGWILGNPECESLFTKESWSYIAETPNPENGTCYPGYFAD
A/Beijing/262/1995 APLQLGNCSVAGWILGNPECESLISKESWSYIVETPNPENGTCYPGYFAD
A/New Caledonia/20/1999 APLQLGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCYPGYFAD
A/North Carolina/3/2003 APLQLGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCYPGYFAD
A/North Carolina/U306-0365/2007 APLQLGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCYPGYFAD
A/Solomon Islands/03/2006 APLQLGNCSVAGWILGNPECELLISRESWSYIVEKPNPENGTCYPGHFAD
A/North Carolina/AF1292/2008 APLQLGNCSVAGWILGNPECELLISKESWSYIVEKPNPENGTCYPGHFAD
A/California/07/2009 APLHLGKNCIAGWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFID
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133a

A/USSR/90/1977 YEELREQLSSVSSFERFEIIPKERSWPKHNVTGVTASC SHKGRSSFYRN 150
A/Kawasaki/6/1986 YEELREQLSSVSSFERFEIIPKESWPNHTVTGVTASC SHKGRSSFYRN
A/Massachusetts/1/1990 YEELREQLSSVSSFERFEIIPKESWPNHTVTGVTASC SHNGKSSFYRN
A/Wellington/47/1992 YEELREQLSSVSSFERFEIIPKESWPNHTVTGVTASC SHNGKSSFYRN
A/Florida/2/1993 YEELREQLSSVSSFERFEIIPKESWPNHTVTGVTASC SHNGKSSFYRN
A/Beijing/262/1995 YEELREQLSSVSSFERFEIIPKESWPNHTVTGVTASC SHNGKSSFYRN
A/New Caledonia/20/1999 YEELREQLSSVSSFERFEIIPKESWPHHTVTGVSASC SHNGKSSFYRN
A/North Carolina/3/2003 YEELREQLSSVSSFERFEIIPKESWPHHTVTGVSASC SHNGKSSFYRN
A/North Carolina/U306-0365/2007 YEELREQLSSVSSFERFEIIPKESWPNHTVTGVSASC SHNGKSSFYRN
A/Solomon Islands/03/2006 YEELREQLSSVSSFERFEIIPKESWPNHTTTGVSASC SHNGESSFYKN
A/North Carolina/AF1292/2008 YEELREQLSSVSSFERFEIIPKESWPNHTVTGVSASC SHNGESSFYRN
A/California/07/2009 YEELREQLSSVSSFERFEIIPKTSWPNHDSNKVTAACPHAGAKSFYKN
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190-helix

A/USSR/90/1977 LLWLTEKNGSYPNLSKSYVNNKEKEVLVLWGVHHPNIEDQKTIYRKENA 200
A/Kawasaki/6/1986 LLWLTEKNGSYPNLSKSYVNNKEKEVLVLWGVHHPNIRDQAIYHTENA
A/Massachusetts/1/1990 LLWLTEKNGLYPNLSKSYVNNKEKEVLVLWGVHHPNIGDQAIYHTENA
A/Wellington/47/1992 LLWLTKKNGLYPNLSKSYVNNKEKEVLVLWGVHHPNIGDQAIYHTENA
A/Florida/2/1993 LLWLTEKNGLYPNLSKSYVNNKEKEVLVLWGVHHPNMGDQAIYHTENA
A/Beijing/262/1995 LLWLTEKNGLYPNLSNSYVNNKEKEVLVLWGVHHPNIRDQAIYHTENA
A/New Caledonia/20/1999 LLWLTKKNGLYPNLSKSYVNNKEKEVLVLWGVHHPNIGDQALYHTENA
A/North Carolina/3/2003 LLWLTKKNGLYPNLSKSYVNNKEKEVLVLWGVHHPNIGDQALYHTENA
A/North Carolina/U306-0365/2007 LLWLTKKNGLYPNLSKSYANNKEKEVLVLWGVHHPNIGDQALYHTENA
A/Solomon Islands/03/2006 LLWLTKKNGLYPNLSKSYANNKEKEVLVLWGVHHPNIGDQALYHTENA
A/North Carolina/AF1292/2008 LLWLTKKNGLYPNLSKSYANNKEKEVLVLWGVHHPNIGDQALYHTENA
A/California/07/2009 LIWLTKKNGSYPNLSKSYINDKGEVLVLWGVHHPNIGDQALYHTENA
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226

A/USSR/90/1977 YVSVVSSNYNRRFTPEIAERPKVRDQAGRINYWTLLPEGDTIIFEANGN 250
A/Kawasaki/6/1986 YVSVVSSHYNRRFTPEIAKRPKVRDQAGRINYWTLLPEGDTIIFEANGN
A/Massachusetts/1/1990 YVSVVSSHYSRRFTPEIAKRPKVRDQAGRINYWTLLPEGDTIIFEANGN
A/Wellington/47/1992 YVSVVSSHYSRRFTPEIAKRPKVRDQAGRINYWTLLPEGDTIIFEANGN
A/Florida/2/1993 YVSVVSSHYSRRFTPEIAKRPKVRDQAGRINYWTLLPEGDTIIFEANGN
A/Beijing/262/1995 YVSVVSSHYSRRFTPEIAKRPKVRDQAGRINYWTLLPEGDTIIFEANGN
A/New Caledonia/20/1999 YVSVVSSHYSRRFTPEIAKRPKVRDQAGRINYWTLLPEGDTIIFEANGN
A/North Carolina/3/2003 YVSVVSSHYSRRFTPEIAKRPKVRDQAGRINYWTLLPEGDTIIFEANGN
A/North Carolina/U306-0365/2007 YVSVVSSHYSRRFTPEIAKRPKVRDQAGRINYWTLLPEGDTIIFEANGN
A/Solomon Islands/03/2006 YVSVVSSHYSRRFTPEIAKRPKVRDQAGRINYWTLLPEGDTIIFEANGN
A/North Carolina/AF1292/2008 YVSVVSSHYSRRFTPEIAKRPKVRDQAGRINYWTLLPEGDTIIFEANGN
A/California/07/2009 YVSVVSSHYSRRFTPEIAKRPKVRDQAGRINYWTLLPEGDTIIFEANGN
YVSVVSSSRYSKPKPEIAIRPKVRDQEGRMNYWTLLPEGDKITFEATGN
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A/USSR/90/1977 LIAPWHAFALNRGFGSG 267
A/Kawasaki/6/1986 LIAPWYAFALSRGFGSG
A/Massachusetts/1/1990 LIAPWYAFALSRGLGSG
A/Wellington/47/1992 LIAPWYAFALSRGFGSG
A/Florida/2/1993 LIAPRYAFALSRGFGSG
A/Beijing/262/1995 LIAPWYAFALSRGFGSG
A/New Caledonia/20/1999 LIAPWYAFALSRGFGSG
A/North Carolina/3/2003 LIAPRYAFALSRGFGSG
A/North Carolina/U306-0365/2007 LIAPRFALSRGFGSG
A/Solomon Islands/03/2006 LIAPRYAFALSRGFGSG
A/North Carolina/AF1292/2008 LIAPRYAFALSRGFGSG
A/California/07/2009 LVVPRYAFAMERNAGSG
* *

Figure S1. Sequence alignment of H1 influenza virus HA1s from 1977-2009 used in this study, Related to Figure 1. Residues of the RBS are highlighted in yellow with other key residues highlighted and marked. See also Figure 1.

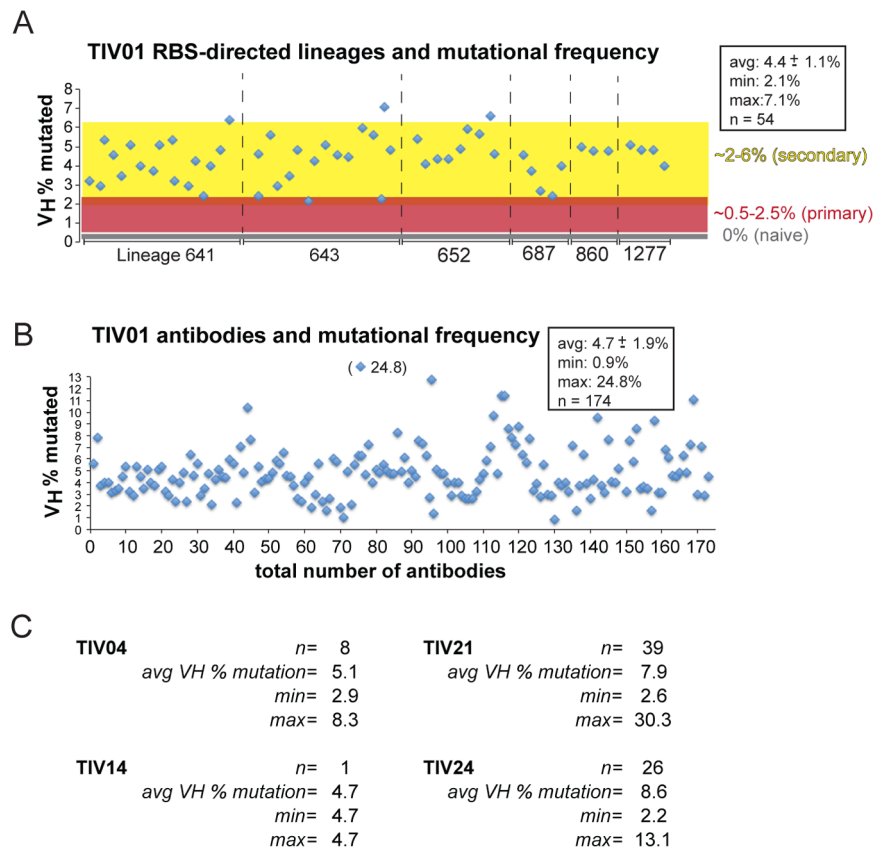
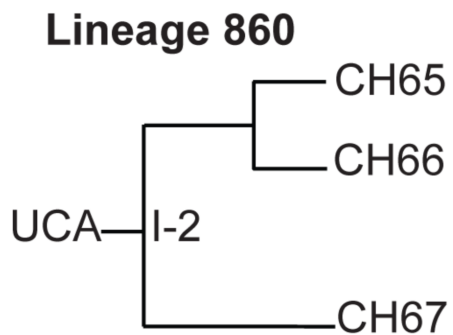


Figure S2. Mutational frequency of isolated TIV antibodies, Related to Figure 2, Figure 3 and Figure 4. (A) Average V_H mutational frequency of RBS-directed lineages. For reference, the approximate mutational frequencies expected for naïve, primary and secondary responses are listed. **(B)** Average V_H mutational frequency of all HA-specific antibodies isolated from TIV01 day 7 plasmablasts. **(C)** Average V_H mutational frequency of all HA-specific antibodies isolated from additional TIV cohort (Moody et al, 2011). See also **Figure 2, Figure 3 and Figure 4.**

	Massachusetts	Solomon Islands
	Δ K133a	+K133a
Lin 641 UCA	>100	1.9
Lin 643 UCA	>100	>100
Lin 652 UCA	>100	>100
Lin 687 UCA	>100	3.6
Lin 860 UCA	>100	59
Lin 1277 UCA	>100	2.2

Figure S3. Dependence of UCA binding on residue K133a, Related to Figure 1 and Figure 5. Affinities (in μ M) for UCAs with deletion (Δ) in H1 Massachusetts (1990) or insertion (+) in H1 Solomon Islands (2006). See also Figure 1 and Figure 5.

A



B

	UCA	I-2
CALIFORNIA (09)	>100	>100
NORTH CAROLINA (08)	>100	>100
NORTH CAROLINA (07)	>100	>100
SOLOMON ISLANDS (06)	>100	>100
NORTH CAROLINA (03)	>100	>100
NEW CALEDONIA (99)	>100	>100
BEIJING (95)	32.6	>100
FLORIDA (93)	34	13.7
WELLINGTON (92)	>100	42
MASSACHUSETTS (90)	15.3	1.3
KAWASAKI (86)	>100	>100
USSR (77)	15.6	>100

Figure S4. Related to Figure 2 and Figure 6. Lineage 860 UCA and I-2 HA-reactivity. (A) Lineage 860 clonal tree; **(B)** data for UCA and I-2 binding to members of the panel of H1 HA1s. K_D values, expressed in mM, are for interaction of monomeric Fab and HA head. See also **Figure 2** and **Figure 6**.