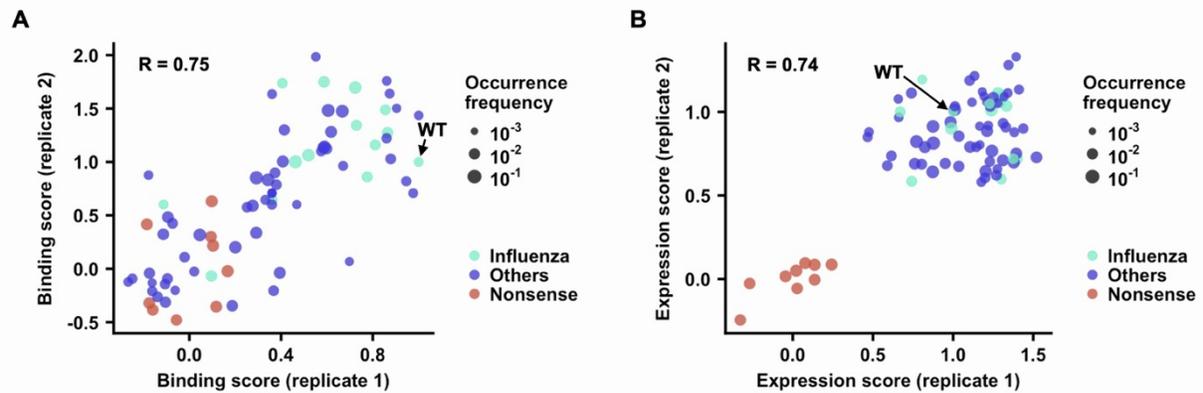


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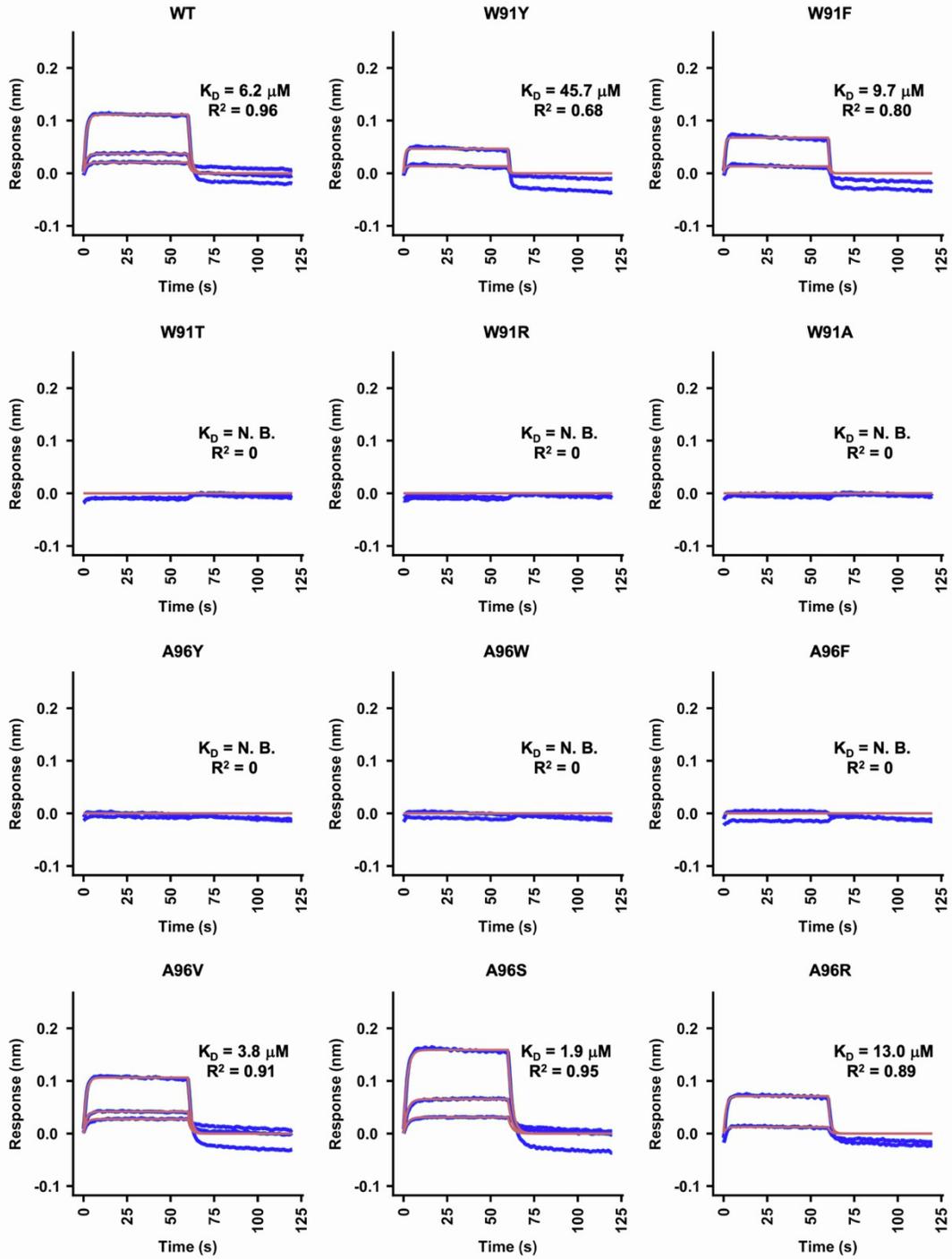
**Supplemental information**

**Stringent and complex sequence constraints  
of an IGHV1-69 broadly neutralizing  
antibody to influenza HA stem**

**Qi Wen Teo, Yiquan Wang, Huibin Lv, Timothy J.C. Tan, Ruipeng Lei, Kevin J. Mao, and Nicholas C. Wu**



**Figure S1. Correlation between biological replicates of binding and expression sorts of CR9114 light chain variant library, Related to Figure 1. (A)** Correlation of binding scores between two independent biological replicates is shown. **(B)** Correlation of expression scores between two independent biological replicates is shown. The datapoint corresponding to CR9114 WT light chain is labeled as “WT”. Cyan: light chain variants from known IGHV1-69 antibodies to influenza virus. Blue: light chain variants from IGHV1-69 antibodies to non-influenza antigens. Red: negative control variants with premature stop codons. The Pearson correlation coefficient (R) is indicated.

**A****mini-HA**

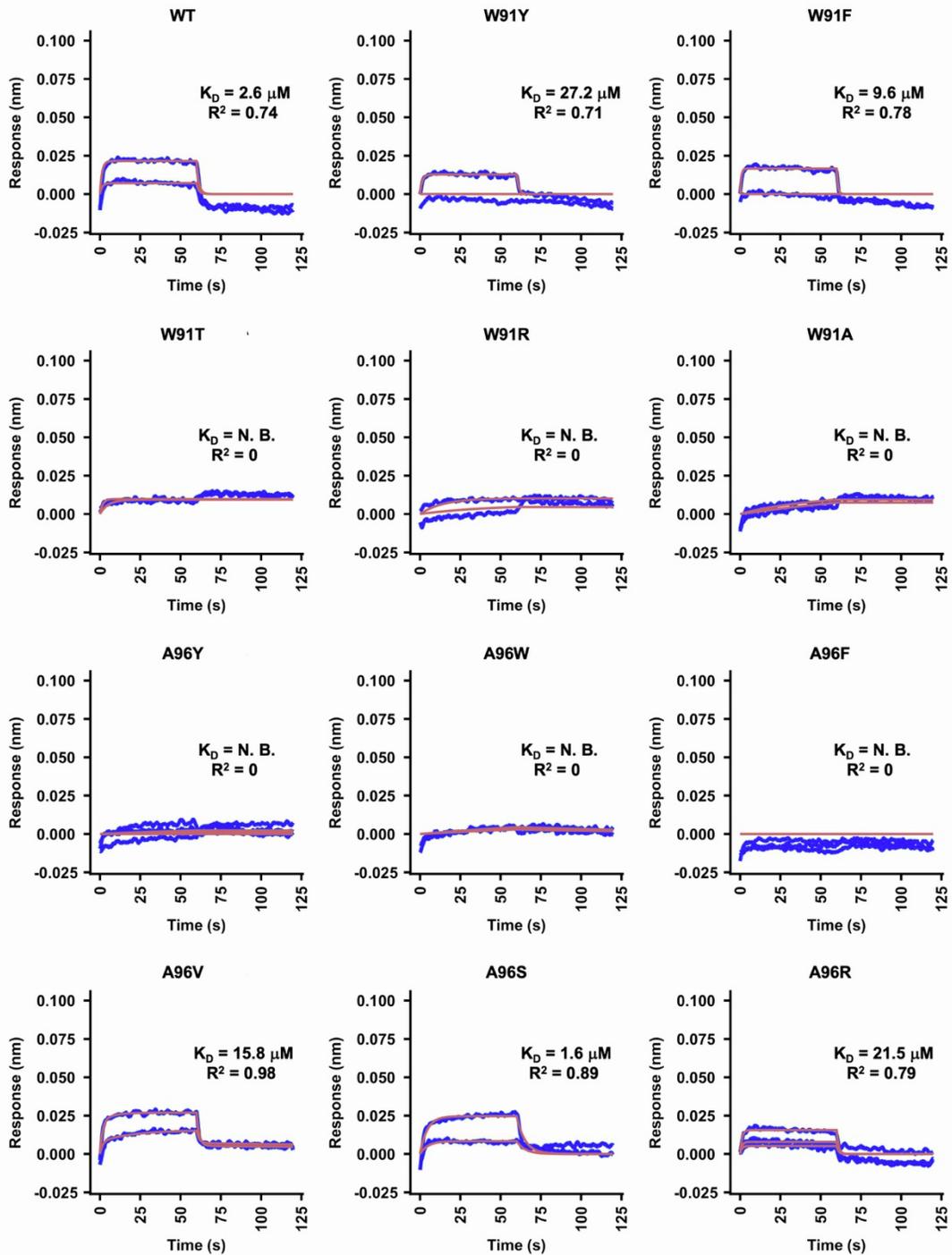
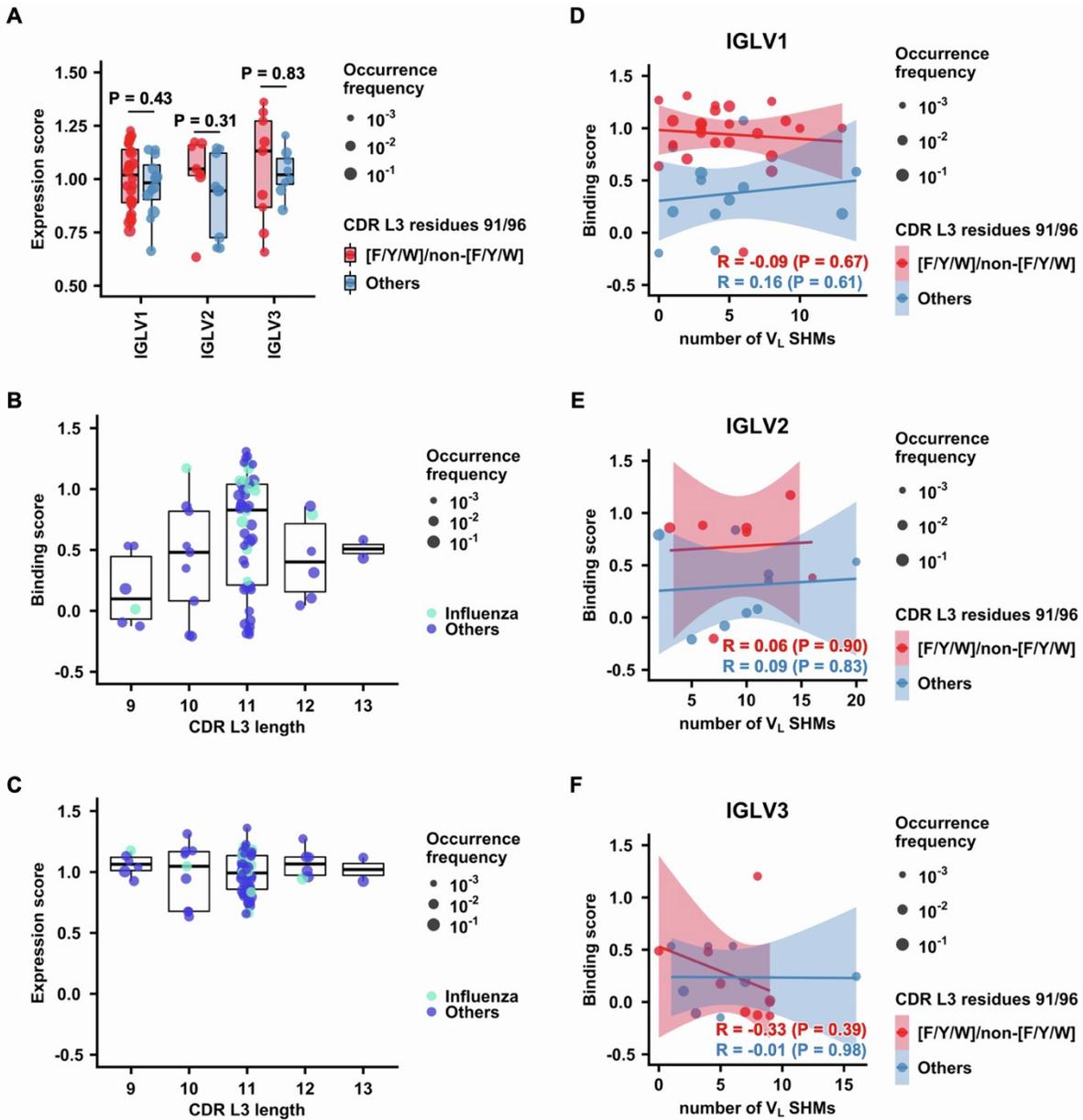
**B****SI06**

Figure S2. Sensorgrams for binding of CR9114 gHC mutants to mini-HA and SI06 HA, Related to Figure 2 and Table 1. Binding kinetics of different Fabs against (A) mini-HA and (B)

SI06 were measured by biolayer interferometry (BLI). Y-axis represents the response. Blue lines represent the response curve and red lines represent the best fit model (1:1 binding model or 2:1 heterogeneous ligand model, see STAR Methods). Binding kinetics were measured for two to three Fab concentrations (33 nM, 100 nM and 300 nM). Dissociation constant ( $K_D$ ) and the goodness of model fitting ( $R^2$ ) are indicated. N.B. indicates no binding.



**Figure S3. Impact of CDR L3 length and somatic hypermutation (SHM) on HA stem binding activity of CR9114, Related to Figure 2. (A)** Expression scores of light chain variants in different IGLV families with and without V<sub>L</sub> 91<sub>F/Y/W</sub>/96<sub>non-F/Y/W</sub> are compared. Red: with V<sub>L</sub> 91<sub>F/Y/W</sub>/96<sub>non-F/Y/W</sub>; Blue: without V<sub>L</sub> 91<sub>F/Y/W</sub>/96<sub>non-F/Y/W</sub>. P-values were computed by two-tailed Student's t-test. **(B-C)** Binding **(B)** and expression **(C)** scores of light chain variants with different CDR L3 lengths are compared. Cyan: light chain variants from known IGHV1-69 antibodies to influenza virus. Blue:

light chain variants from IGHV1-69 antibodies to non-influenza antigens. **(D-F)** Correlation between binding score and the number  $V_L$  SHM is shown for light chain variants in different light chain families, namely IGLV1 **(D)**, IGLV2 **(E)**, and IGLV3 **(F)**. The Pearson correlation coefficient **(R)** is indicated. P-values were computed by Pearson correlation test.

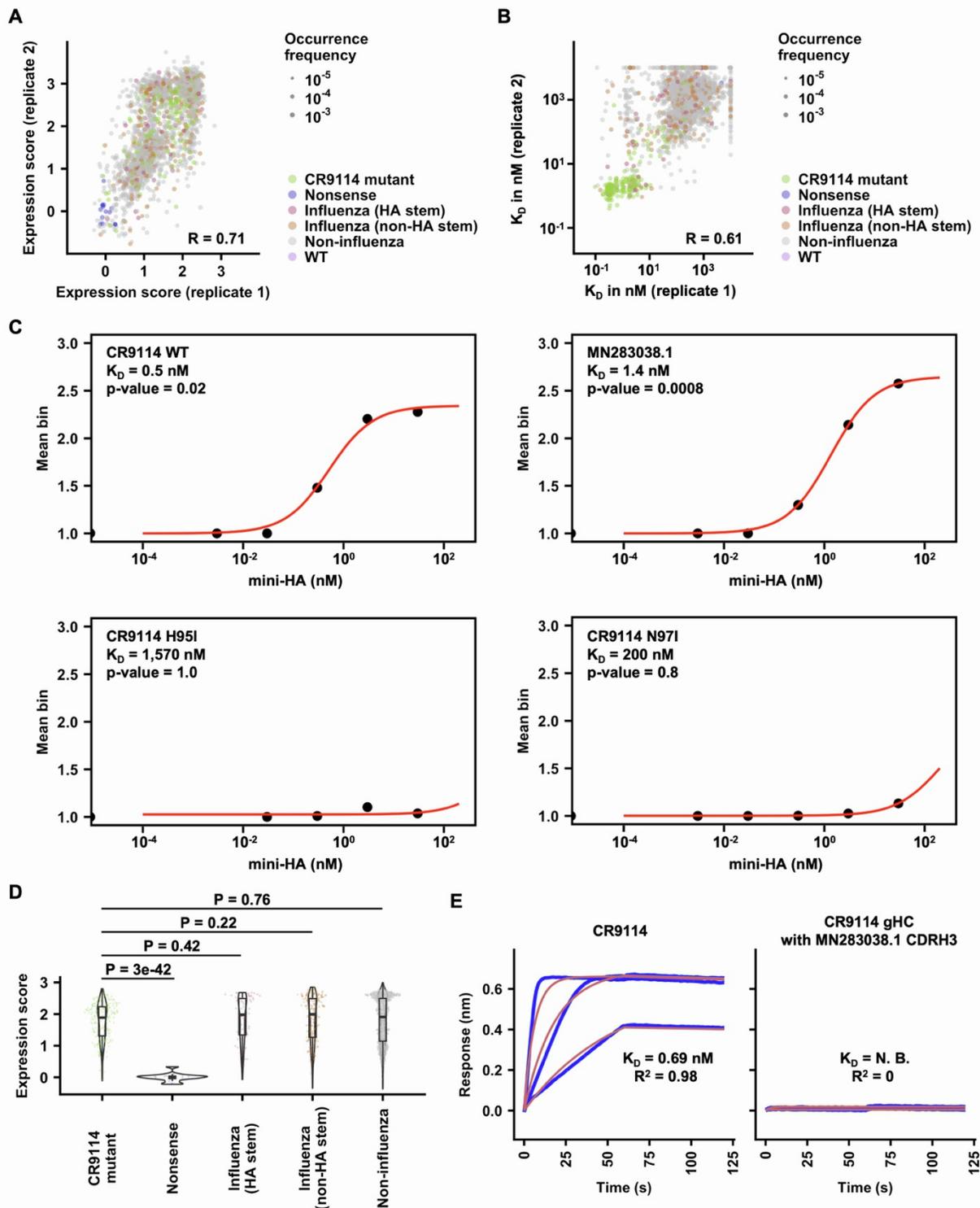
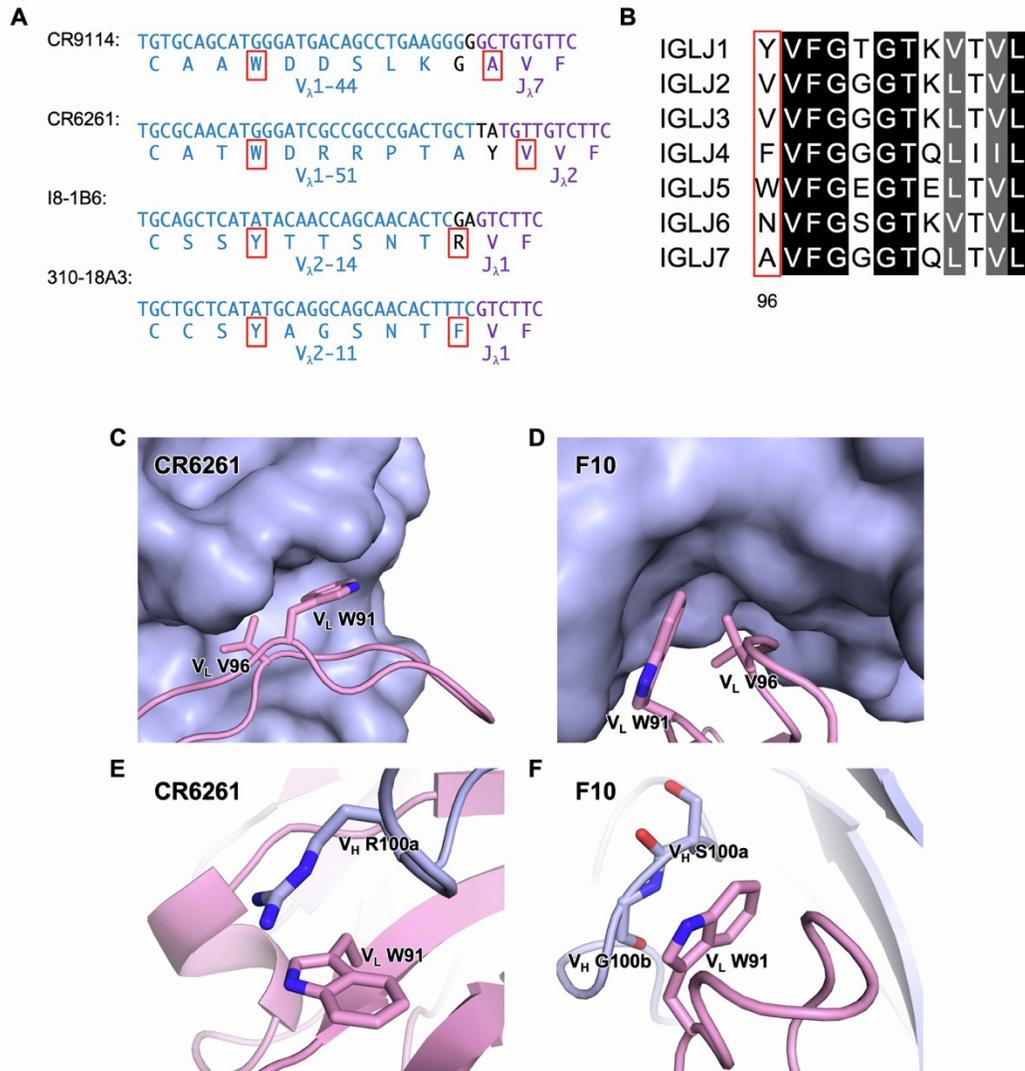


Figure S4. Reproducibility and analysis of Tite-Seq for CDR H3 variants, Related to Figure

3. (A) Correlation of expression scores between two independent biological replicates is shown.

**(B)** Correlation of apparent dissociation constant ( $K_D$ ) values between two independent biological replicates is shown. **(C)** Example titration curves inferred from Tite-Seq data. **(D)** Distributions of expression scores for CDR H3 variants from different types of antibodies. Greenish yellow: CR9114 single amino acid mutants; purple: nonsense variants with stop codons; red: CDR H3 variants from IGHV1-69 HA stem antibodies; orange: CDR H3 variants from IGHV1-69 non-HA stem influenza antibodies; grey: CDR H3 variants from IGHV1-69 non-influenza antibodies; pink: WT. **(E)** Binding kinetics of a CDR H3 variant from an IGHV1-69 non-influenza antibody (Genbank ID: MN283038.1) and CR9114 (positive control) to mini-HA were measured by BLI. Y-axis represents the response. Blue lines represent the response curve and red lines represent a 1:1 binding model. Binding kinetics were measured for at least two Fab concentrations. N.B. indicates no binding. Dissociation constant ( $K_D$ ) and the goodness of model fitting ( $R^2$ ) are indicated.



**Figure S5. Additional characterizations of IGHV1-69 HA stem antibodies, Related to Figure 4.** (A) Nucleotide and amino acid sequences of light chain V-J junction are shown for different IGHV1-69 HA stem antibodies. V<sub>L</sub> residues 91 and 96 are indicated in red. Blue: V-region; purple: J-region; black: N-region. (B) CDR L3 sequences among different IGLJ families. V<sub>L</sub> residue 96 is indicated in red. (C-D) Side chains of V<sub>L</sub> W91 and A96 at the heavy-light chain interfaces of (C) CR6261 and (D) F10 are shown as sticks representation. Heavy chain is in light blue surface representation. Light chain is colored in pink. (E)  $\pi$ - $\pi$  stacking interaction between V<sub>H</sub> R100a and V<sub>L</sub> W91 of CR6261 is shown. (F)  $\pi$ - $\pi$  stacking interaction between V<sub>H</sub> S100a-G100b peptide bond

and  $V_L$  W91 of F10 is shown. PDB 3GBN [S1] and PDB 3FKU [S2] are used for CR6261 and F10, respectively.  $V_H$  and  $V_L$  indicate variable regions of antibody heavy and light chains, respectively.

**Table S4. List of primers used in this study, Related to STAR Methods.**

<b>Primer Name</b>	<b>Sequence (5' to 3')</b>
V <sub>H</sub> 1-69-Lightchain-lib-VF	GGACAACCAAAGGCTGCTCCTTC
V <sub>H</sub> 1-69-LightChain-lib-VR	GGCCGGCTGGGCCGCTGCTAAACTGA
V <sub>H</sub> 1-69-LightChain-lib-IF	TTTCAATATTTTCTGTTATTGCTTCAGTTTTAGCAGCGGCCAGCCGGCC
V <sub>H</sub> 1-69-LightChain-lib-IR	TCAGAGGATGGAGGGAACAAGGTGACAGAAGGAGCAGCCTTTGGTTGTCC
V <sub>H</sub> 1-69-LightChain-recover-F	CAGTTTTAGCAGCGGCCAGCCG
V <sub>H</sub> 1-69-LightChain-recover-R	ACAGAAGGAGCAGCCTTTGGTTG
V <sub>H</sub> 1-69-CDRH3-VF	GGCCAAGGGACCACGGTCACCGTCTCCTCAGCTTC
V <sub>H</sub> 1-69-CDRH3-VR	GTAATACACGGCCGTGTCCTCAGATCTCAGGCTGC
V <sub>H</sub> 1-69-CDRH3-lib-F	CACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGATTAC
V <sub>H</sub> 1-69-CDRH3-lib-R	AAAACGGAAGGTCCCTTAGTAGAAGCTGAGGAGACGGTGACCGTGGTCCCTTGGC
V <sub>H</sub> 1-69-CDRH3-recover-F	ATCTGAGGACACGGCCGTGATTAC
V <sub>H</sub> 1-69-CDRH3-recover-R	AGACGGTGACCGTGGTCCCTTGGCC

## Supplemental References

- S1. Ekiert, D.C., Bhabha, G., Elsliger, M.A., Friesen, R.H., Jongeneelen, M., Throsby, M., Goudsmit, J., and Wilson, I.A. (2009). Antibody recognition of a highly conserved influenza virus epitope. *Science* 324, 246-251. [10.1126/science.1171491](https://doi.org/10.1126/science.1171491).
- S2. Sui, J., Hwang, W.C., Perez, S., Wei, G., Aird, D., Chen, L.M., Santelli, E., Stec, B., Cadwell, G., Ali, M., et al. (2009). Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. *Nat Struct Mol Biol* 16, 265-273. [10.1038/nsmb.1566](https://doi.org/10.1038/nsmb.1566).