

Supplementary material

Structural Analysis of the Unmutated Ancestor of the HIV-1 Envelope V2 Region Antibody CH58 Isolated from an RV144 Vaccine Efficacy Trial Vaccinee

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The opinions herein are those of the authors and should not be construed as representing the official views of the Departments of Defense or the Army.

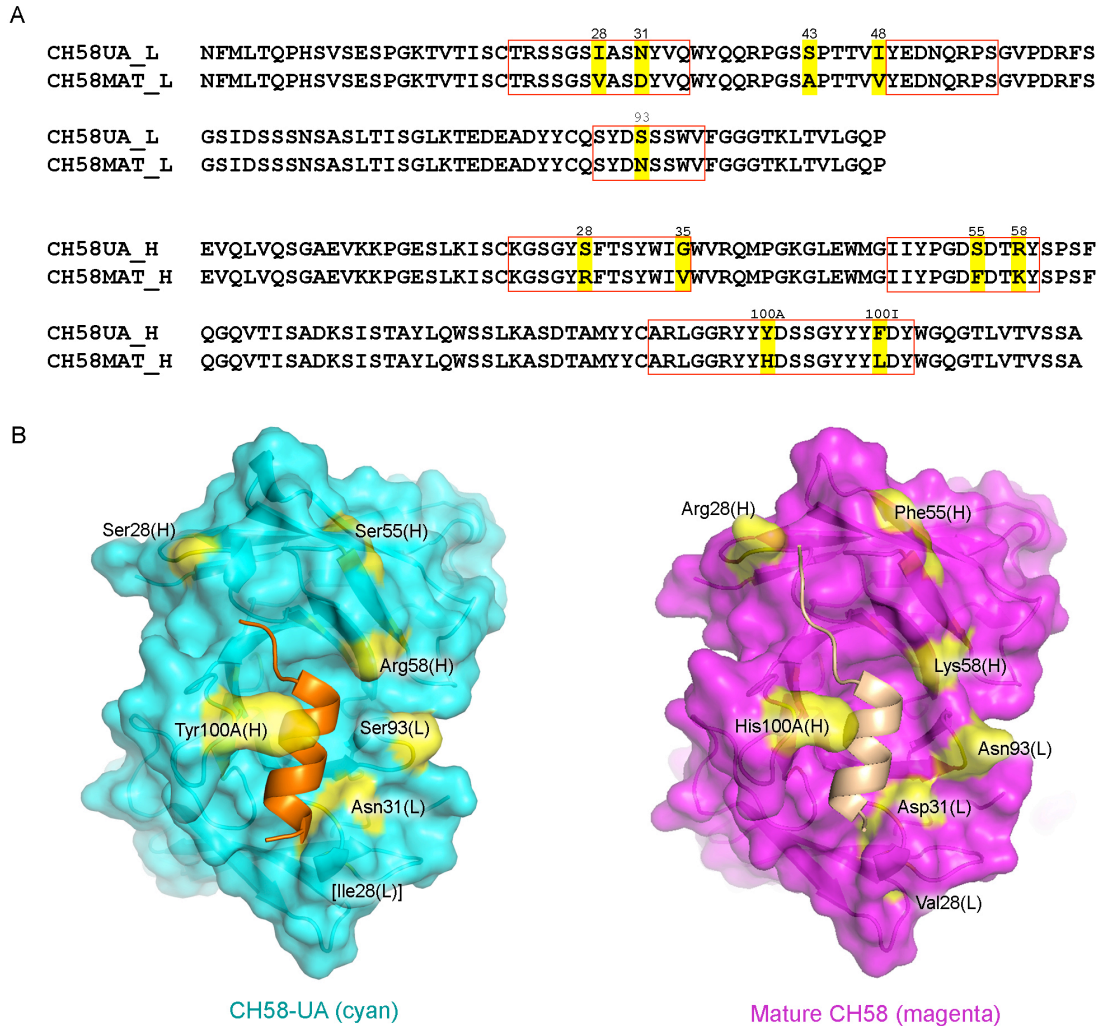


Figure S1 Sequence alignments and locations of mutations in the mature CH58 antibody (“CH58MAT”) and its inferred unmutated ancestor (“CH58UA”). A) The light and Fab heavy chains of the CH58-UA and mature CH58 antibodies have a total of 11 mutations (highlighted in yellow and numbered) that occur with affinity maturation. Nine of those mutations occurred in complementarity determining regions (boxed). B) Surface representations of the structures of CH58-UA and mature CH58 (Liao et al., 2013) Fabs with gp120 V2 peptides show that some but not all the mutations occur within the antibody paratope. Residues visible on the surface of the Fabs are highlighted in yellow and labeled.

Table S1 Summary data on BLI binding experiments between CH58-UA Fab and wild-type V2 peptide gp120₁₆₅₋₁₈₂.

	1	2	avg
k_a	$1.8 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$	$1.4 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$	$1.6 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$
k_d	$1.6 \times 10^{-1} \text{ s}^{-1}$	$1.8 \times 10^{-1} \text{ s}^{-1}$	$1.7 \times 10^{-1} \text{ s}^{-1}$
K_D	9.0 μM	12.9 μM	11.0 μM

Table S2 Summary data on BLI binding experiments between mature CH58 Fab and wild-type V2 peptide gp120₁₆₅₋₁₈₂.

	1	2	3	avg
k_a	$6.5 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$	$6.6 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$	$6.7 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$	$6.6 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$
k_d	$2.7 \times 10^{-4} \text{ s}^{-1}$	$3.1 \times 10^{-4} \text{ s}^{-1}$	$3.3 \times 10^{-4} \text{ s}^{-1}$	$3.0 \times 10^{-4} \text{ s}^{-1}$
K_D	4.2 nM	4.7 nM	4.9 nM	4.6 nM

Table S3 Summary data on BLI binding experiments between CH58-UA Fab and V2 peptide gp120₁₆₅₋₁₈₂ L179A/I181A.

	1	2	avg
k_a	$7.2 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$	$9.5 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$	$8.4 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$
k_d	$7.4 \times 10^{-2} \text{ s}^{-1}$	$6.9 \times 10^{-2} \text{ s}^{-1}$	$7.2 \times 10^{-2} \text{ s}^{-1}$
K_D	1.0 μM	0.7 μM	0.9 μM

Reference:

Liao, H.X., Bonsignori, M., Alam, S.M., McLellan, J.S., Tomaras, G.D., Moody, M.A., Kozink, D.M., Hwang, K.K., Chen, X., Tsao, C.Y., *et al.* (2013). Vaccine induction of antibodies against a structurally heterogeneous site of immune pressure within HIV-1 envelope protein variable regions 1 and 2. *Immunity* 38, 176-186.